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PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD

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I.—INTRODUCTION

Agronomists have long recognized the fact that the plots of an experimental field may differ considerably among themselves. This variability is the source of the greatest difficulty in the interpretation of comparative cultures. A recent analysis (*3*)[1] of the available data by adequate biometric formulae (*1*) has shown that heterogeneity is a practically universal characteristic of experimental fields and that it must be considered in the interpretation of the results of all plot tests.

With the demonstration of this characteristic of experimental areas the questions naturally arise: Are the differences between plots transient or are they relatively permanent from year to year? Do these differences tend to increase or to decrease with cultivation?

Presumably the differences which obtain in the soil of an experimental field are in part permanent and in part transient. Lyon (*5*) suggested that tillage and other factors will change the plots so that the results will not be comparable from year to year. Unfortunately he does not present data to show to what extent this may be true. He gives a series of yields for successive years on the same plots, which measured 33 by 66 feet or $^1/_{20}$ of an acre in area, at the Nebraska Agricultural Experiment Station and shows that the rank of the yield of these plots differs greatly from year to year. Thus he concludes that if they differ among themselves in their capacity for crop production this difference is very little constant from year to year.

Smith (*6*) took advantage of the breaking up of a piece of land which had lain 16 years in pasture to investigate the effect of cultivation on the uniformity of a series of plots. Any influence of 1 or 2 years preceding cultures on the variation or correlation of yields should, he assumed, be apparent in the statistical constants deduced from these

[1] Reference is made by number (italic) to "Literature cited," p. 356.

data. He gives a table which indicates that there is such a change. He says:

> It is noticeable that the variability as measured by the standard deviation becomes less in each succeeding year. This suggests the question as to whether continued cropping might not tend to induce uniformity. The records of a few of these plots which were continued in corn for three years longer do not support such a conclusion.

It must be noted that in Smith's experiments seasonal conditions varied greatly from year to year. Thus 1895, which was exceedingly dry and also cool in the early part of the season, was highly unfavorable. The two following years were unusually favorable for corn. As a result the yields were, respectively, 31.6, 91.6, and 71.4 bushels per acre in the three years.

Lehmann in his work at the experimental farm near Bangalore attempted to use the experience of previous years in the standardization of experimental plots. His data will be considered in some detail below.

II.—METHODS AND RESULTS

The permanency of the differentiation of plots in their capacity for crop production may be measured in terms of correlation. If the plots of a field differ among themselves in a more or less permanent way there will, with reasonably uniform climatic conditions, be a correlation between the yields of the plots of a series in two or more successive years—in short, an intera nnual correlation (*2*).

The problem of the correlations between the yields of identical plots in different years is one of very great interest. If this correlation be high it should be possible to standardize a field of plots by one or more sowings to the same variety. A chief difficulty in the standardization of the field by the carrying out of a preliminary test in which the productive capacities of the plots are determined once and for all lies in the fact that the factors which determine yield are in part edaphic—that is, pertaining to soil conditions—and in part meteorological. For example, in a very dry year sections of a field which are lower may produce the heaviest crops because adequate moisture is longer retained in these places. In a wet year the case may be just the reverse, for the crops in the lower-lying portions may be too wet for the best plant growth. Thus, it is quite possible that in cases in which there is a profound influence of environmental factors there may be a negative correlation between the yield of the same plots in different years.

It is conceivable, therefore, that the interannual correlation for yield per plot may range from negative to positive values, zero correlation being found in cases in which edaphic and meteorological factors exactly counterbalance each other in their influence upon the yield of the plots of a heterogeneous field.

A.—PUBLISHED DATA

Unfortunately few data are available for analysis from the literature. Lehmann has given (*4, p. 6*) yields of paddy on the 17 plots of ranges B and C, respectively, of the wet tract of the Experimental Farm at Hebbel. Grouping the yields for the two ranges, we find for the correlations between the yields of the same plots in the two years 1905 and 1906:

Range B, $r = 0.834 \pm 0.050$, $r/Er = 16.7$.
Range C, $r = 0.799 \pm 0.059$, $r/Er = 13.5$.

Stockberger (7) gives data for the extremes of a series of hill yields for hops. The interannual correlations deduced from these data have been shown (*2*) to be as follows:

Years.	Lowest hills.	Highest hills.
1909 and 1910	0.29±0.17	0.59±0.13
1910 and 1911	.55± .13	.52± .14
1909 and 1911	.43± .15	.30± .18

Stockberger has also given (*8*) the yields for 30 rows, each 210 feet in length, from hop fields of several hundreds of acres in the Sacramento Valley of California:

> The plants in these rows averaged well in number and uniformity of growth with the plants on several hundreds of acres of hops in the midst of which the experimental area was located.

Data are available for the years 1909 to 1914. Calculating the correlation between the yields in the different years, we have the results set forth in Table I. It appears that with one single exception the constants are positive throughout. In general they are significant in comparison with their probable errors, indicating a superiority in a subsequent year if a superiority is shown in a given year.

The constants in the table are arranged in a way to show the change in the coefficient of correlation as the years become more widely separated in time. Thus, in the case of the correlation for the 1909 yields, the constant for "first and second" is that showing the relationship between the 1909 and 1910 yields, while "first and third" indicates the constant measuring the relationship between the yields of 1909 and 1911. Similarly, in the series of coefficients for 1910 "first and second" designates the correlation between 1910 and 1911, etc.

For the series beginning with 1909 we note a marked decrease in the magnitude of the constants as the yields correlated become more widely separated in time. The same is true for the series beginning with 1910. The other series are more irregular.

TABLE I.—*Interannual correlations for yield of hops*

Beginning of series.	First and second years.	First and third years.	First and fourth years.	First and fifth years.	First and sixth years.
1909	+0.768±0.051	+0.622±0.075	+0.380±0.105	+0.259±0.115	+0.061±0.123
1910	+ .577± .082	+ .447± .099	+ .451± .098	+ .274± .114	
1911	+ .062± .123	+ .313± .111	− .126± .121		
1912	+ .311± .111	+ .705± .062			
1913	+ .597± .079				

The most reasonable explanation of the higher correlation of more closely associated years is that both field conditions and the productiveness of the individual vines change more or less as time goes on. The result of such changes would be a lower correlation between the yields of periods more widely separated in time.

The data for the dry-land experiments in Mysore State have been discussed elsewhere (3) in relation to the problem of field heterogeneity. It was shown there that in two dry years the field showed marked hetereogeneity, but that in one unusually wet season there was marked abnormality of yield with little correlation between the yields of adjacent plots.

It seems of unusual interest, therefore, to determine to what extent the differences between these plots are permanent from year to year. Correlating between the yields of ragi, we find the following correlation coefficients for the whole series of 105 plots for which data are available.

Years.	Grain.	Straw.	Total.
1905 and 1906	0.591±0.043	0.777±0.026	0.757±0.028
1905 and 1907	.693± .034	.855± .018	.852± .018
1906 and 1907	.450± .052	.678± .036	.610± .041

The correlations are of very substantial order, and without exception they are clearly significant in comparison with their probable errors. They show that the differences in the plots are to a high degree persistent during the three years of this experiment.

For grain, straw, and total yield the correlations between the yield for 1905 and 1907 are higher than those for 1905 and 1906 or for 1906 and 1907. If there were a progressive change in the field one might have expected that the correlations would be higher between consecutive years. Apparently the influence of the abnormal conditions of 1906 has been to lower the correlations for this year.

The results show that the capacity for production is to a high degree persistent from year to year, notwithstanding great diversity in meteorological conditions.

A series of records of unusual interest is provided by Smith (6) for yields of corn in three successive years, 1895, 1896, 1897. It has been

shown elsewhere (3) that this field, which had lain in grass for many years, is highly heterogeneous, showing correlations between adjacent plots of $r=0.61$ to $r=0.83$.

The conditions for corn production differed very greatly in the three years. Thus the constants for yield were:

Year.	Mean.	Standard deviation.	Coefficient of variation.
1895	31.7	7.91	25.0
1896	91.6	10.64	11.6
1897	71.4	6.27	8.8

Yield is over twice as heavy in the second and third years as in the first. The variability in yield as measured by the coefficient of variation is far lower in the second and third years than in the first.

Computing the correlations between the yields for the three years, we have the following results:

For 1895 and 1896, $r=-0.354\pm0.054$, $r/E_r=6.6$.

For 1895 and 1897, $r=-0.221\pm0.059$, $r/E_r=3.8$.

For 1896 and 1897, $r=+0.818\pm0.020$, $r/E_r=40.1$.

There is a negative correlation for 1895 and 1896 and for 1895 and 1897 but a high positive correlation for 1896 and 1897. Thus the plots which were better in the highly unfavorable year 1895 were poorer in the two favorable years 1896 and 1897. Plots which were better in the favorable year 1896 were also better in the favorable year 1897.

B.—THE HUNTLEY UNIFORM CROPPING EXPERIMENT

The most extensive series of records available is that for a uniform cropping experiment conducted for the past several years at the Field Station of the Office of Western Irrigation Agriculture, at Huntley, Mont.

The Huntley field lies in the Yellowstone Valley on land having a very slight and uniform slope to the north. The detailed history of the field prior to 1910 is not known definitely. It was probably first broken from the native prairie sod in the spring of 1908. In 1909 it was planted to sugar beets, but the crop was destroyed in the late summer. It came under experimental control in 1910, when the major portion of it was sown to oats and yielded a crop of 66 bushels per acre. In that season a small tract in the northeast corner of the field was used as a machinery park or stack yard and was not put into crop. This tract occupied about two-thirds of the length of the first five plots in series II. It is possible that this difference of treatment in 1910 may have been reflected in the crop yields of 1911, but it seems hardly probable that any material effects could have persisted longer.

In the spring of 1911 this field was laid out into 46 plots, each measuring 23½ by 317 feet and containing 0.17 acre, arranged in two parallel series of 23 plots each. The two series of plots were separated merely by a temporary irrigation ditch. In 1911 it was planted to sugar beets, and in the spring of 1912 it was seeded to alfalfa, and one cutting was harvested that year. This stand remained on the ground during 1913 and 1914, when the entire field was fall-plowed. In 1913 three cuttings were made, but the third cutting was lost in a heavy wind which scattered and mixed the crop before weighings from the various plots could be made. The first cutting, designated as alfalfa I, was made on plots one-half the original size. The second cutting was harvested from plots one-quarter the original size. The first and second cuttings in 1914 were weighed for plots one-quarter the original size—that is, 0.0425-acre plots—while the third cutting was recorded for plots one-third the original size. These furnish the data for alfalfa I, II, and III for 1914. Total yields for the first and second cuttings in 1913 and 1914 and for the first, second, and third cuttings in 1914 are also considered.

In 1915 and 1916 ear corn was grown. In 1917[1] the fields were planted to oats, and records were made of grain, straw, and total yield. In 1918 silage corn was grown. In 1919 the land produced a crop of barley.

It has been the practice each season to treat the whole field as a unit until harvest time, when the plot boundaries are established in order to measure the crop yields. In other words, all cultural operations, including irrigation, are carried out on a field scale and uniformly throughout the field. No manuring has so far been attempted. An effort has been made to avoid any artificial causes of heterogeneity.

The crop yields each year have been satisfactory—that is, they have not been abnormal—as is shown in Table II, where the mean yields per plot and per acre are set down. Fortunately, this experiment has also escaped injury from insect pests, plant diseases, and storms, which so often interfere with the success of long-term field experimentation.

[1] Because of other activities the plots could not be harvested in halves and quarters in 1917–1919.

TABLE II.—*Mean yields of the Huntley uniform cropping experiment*

Crop.	Number of pounds per plot.	Number of tons or bushels per acre.
1911, sugar beets	4,179.00	12.29
1912, total alfalfa	356.54	1.04
1913, alfalfa I	541.41	1.59
1913, alfalfa II	483.26	1.42
1913, alfalfa I and II	1,024.67	3.01
1914, alfalfa I	489.13	1.44
1914, alfalfa II	499.34	1.47
1914, alfalfa I and II	988.47	2.91
1914, alfalfa III	471.95	1.38
1914, alfalfa I to III	1,460.43	4.29
1915, ear corn	522.58	52.7
1916, ear corn	396.15	41.6
1917, oat grain	555.80	102.1
1917, oat straw	521.54	1.53
1917, total yield	1,077.34	3.16
1918, silage corn	3,175.43	9.34
1919, barley grain	358.19	43.8
1919, barley straw	230.50	.67
1919, total yield	588.69	1.73

The data furnished by this series of records are of particular value, since (*a*) they are based on irrigated plots and (*b*) it is possible to compare the correlations between the same crop and different crops in the different years.

The correlations between the yields of the various crops in the different years may be considered in three series.

(1) The first comprises the yields for the whole plots. In this series we determine the correlation between the crop produced on the 46 plots in one year and that produced on the same 46 plots in another year.

(2) In the study of certain crops the plots were divided into two subplots, and we may determine the relationship between yield of individual subplots in different years. Then the number of observations is twice what it was in the preceding correlation, that is, $N=92$ instead of 46.

(3) Finally, in a more limited series of cases the 46 original plots were harvested in 4 subplots each, thus increasing the number of units which may be entered in the correlation tables to 184.

The data for determining the correlations between yields of various crops for the 46 whole plots are given in Table III. The data for half plots and quarter plots may be obtained from the diagrams in an earlier paper by Harris (*3*) on the practical universality of field heterogeneity as a factor affecting plot yields. The correlation coefficients and their probable errors for whole plots are shown in Table IV.

TABLE III.—*Yield of plots of field B at the Huntley (Mont.) Field Station* [a]

Plot No.	1911, sugar beets.	1912, total alfalfa.	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1914, alfalfa III.	1914, alfalfa I to III.
II, 1	12.78	260	595	600	1,195	585	550	1,135	580	1,715
2	12.70	395	530	560	1,090	610	605	1,215	605	1,820
3	10.04	397	640	630	1,270	605	690	1,295	595	1,890
4	10.35	435	640	650	1,290	640	660	1,300	610	1,910
5	9.33	442	625	540	1,165	590	700	1,290	595	1,885
6	9.40	419	625	595	1,220	645	735	1,380	510	1,890
7	11.53	438	640	575	1,215	625	775	1,400	500	1,900
8	12.40	410	555	570	1,125	555	725	1,280	500	1,780
9	10.28	418	570	470	1,040	590	615	1,205	475	1,680
10	11.81	393	540	470	1,010	545	505	1,050	450	1,500
11	13.99	405	585	435	1,020	580	430	1,010	400	1,410
12	12.28	435	530	450	980	555	425	980	445	1,425
13	11.91	385	565	485	1,050	465	445	910	455	1,365
14	13.42	395	555	510	1,065	540	480	1,020	485	1,505
15	12.28	405	655	565	1,220	535	515	1,050	540	1,590
16	13.76	305	650	475	1,125	545	440	985	475	1,460
17	11.73	312	590	435	1,025	540	435	975	460	1,435
18	12.49	290	635	425	1,060	540	525	1,065	455	1,520
19	15.55	315	635	455	1,090	545	490	1,035	465	1,500
20	11.93	310	605	440	1,045	540	505	1,045	475	1,520
21	13.52	330	625	455	1,080	580	535	1,115	510	1,625
22	14.36	325	625	500	1,125	610	525	1,135	520	1,655
23	16.81	310	590	425	1,015	490	445	935	380	1,315
III, 1	13.93	405	535	425	960	420	470	890	645	1,535
2	13.04	350	470	430	900	430	395	825	520	1,345
3	10.55	400	510	405	915	395	435	830	495	1,325
4	11.63	435	475	425	900	440	450	890	440	1,330
5	10.56	350	460	445	905	435	420	855	455	1,310
6	10.00	365	510	510	1,020	430	375	805	415	1,220
7	10.54	390	500	440	940	410	400	810	445	1,255
8	10.00	325	455	425	880	415	380	795	425	1,220
9	8.85	360	490	375	865	425	410	835	385	1,220
10	10.48	360	440	415	855	365	390	755	360	1,115
11	12.61	335	485	390	875	360	420	780	385	1,165
12	11.22	350	470	400	870	360	430	790	370	1,160
13	12.08	370	500	450	950	390	505	895	435	1,330
14	11.91	255	470	485	955	370	495	865	425	1,290
15	12.65	370	485	455	940	380	470	850	435	1,285
16	11.71	325	460	440	900	360	455	815	410	1,225
17	12.19	280	460	445	905	395	425	820	430	1,250
18	12.62	280	430	500	930	395	425	820	385	1,205
19	13.45	320	480	515	995	450	515	965	475	1,440
20	15.60	275	520	565	1,085	435	480	915	445	1,360
21	16.25	290	460	510	970	435	450	915	465	1,380
22	14.70	345	530	530	1,060	475	515	990	495	1,485
23	16.52	337	505	530	1,035	475	475	950	475	1,425

[a] All yields are given in pounds per plot with the exception of that for sugar beets, which is given in tons per acre.

TABLE III.—*Yield of plots of field B at the Huntley (Mont.) Field Station* [a]—Con.

Plot No.	1915, ear corn.	1916, ear corn.	1917, oat grain.	1917, oat straw.	1917, total yield.	1918, silage corn.	1919, barley grain.	1919, barley straw.	1919, total yield.
II, 1	556	513	580	574	1,154	3,655	392	288	680
2	598	514	593	631	1,224	3,285	349	251	600
3	526	481	606	588	1,194	3,290	377	253	630
4	558	495	598	414	1,012	3,390	352	218	570
5	509	487	614	590	1,204	3,570	414	246	660
6	521	450	596	584	1,180	3,240	426	264	690
7	499	489	572	458	1,030	3,005	463	262	725
8	502	441	574	524	1,098	3,010	424	276	700
9	515	434	553	495	1,048	3,060	425	265	690
10	583	415	614	606	1,220	2,885	422	298	720
11	524	399	574	578	1,152	2,955	386	224	610
12	507	379	548	510	1,058	3,055	365	240	605
13	528	376	537	523	1,060	3,125	350	220	570
14	507	372	540	522	1,062	3,210	368	222	590
15	511	398	518	616	1,134	3,155	344	191	535
16	524	409	564	570	1,134	2,870	351	204	555
17	520	389	499	481	980	2,950	333	127	460
18	479	408	538	518	1,056	3,235	309	241	550
19	455	404	637	605	1,242	3,330	313	177	490
20	489	383	579	497	1,076	3,150	364	221	585
21	519	455	567	513	1,080	3,180	376	229	545
22	573	473	553	477	1,030	3,075	306	199	505
23	578	414	509	391	900	3,375	288	257	545
III, 1	545	404	563	547	1,110	3,685	332	238	570
2	552	376	560	522	1,082	3,365	352	218	580
3	504	337	511	511	1,022	3,315	375	260	635
4	547	328	523	497	1,020	3,170	342	183	525
5	544	338	532	516	1,048	3,240	416	284	700
6	533	312	536	552	1,088	3,290	460	250	710
7	505	311	538	544	1,082	2,855	410	330	740
8	519	345	552	556	1,108	2,905	400	260	660
9	513	353	515	535	1,050	2,965	400	260	660
10	509	337	521	545	1,066	2,760	386	274	660
11	493	322	473	479	952	2,640	403	262	665
12	496	357	520	462	982	2,850	305	255	560
13	503	343	645	377	1,022	2,880	290	199	495
14	496	333	525	469	994	3,190	290	130	420
15	518	360	557	485	1,042	3,100	301	174	475
16	499	372	578	504	1,082	2,975	335	185	520
17	483	353	549	515	1,064	2,995	317	188	505
18	469	367	563	517	1,080	3,315	320	190	580
19	477	410	562	512	1,074	3,540	293	187	480
20	490	407	561	481	1,042	3,280	323	177	500
21	551	406	486	450	942	3,370	331	259	580
22	628	423	573	571	1,144	3,625	302	218	520
23	654	401	561	573	1,134	3,705	241	249	490

[a] All yields are given in pounds per plot with the exception of that for sugar beets, which is given in tons per acre.

From the series of correlations as a whole it appears that of the 152 coefficients showing the relationship between crop yields in different years, 133 are positive while only 19 are negative in sign. If the differences in capacity for crop production demonstrated in different years were due to purely transient causes, one would expect to find an approximately equal number of positive and negative correlations with the general average value sensibly zero. Instead we find the proportion of 133 to 19. This is a deviation from the ratio 76 to 76, which one might expect on the assumption that there is no correlation between the yields of plots in a series of years, of

$$57 \pm 0.6745\sqrt{152 \times 5 \times 0.5} = 57 \pm 4.16.$$

The deviation from equality is 13.7 times as large as its probable error and is unquestionably significant.

If we consider that coefficients which are 2.5 times or more as large as their probable errors represent statistically significant interrelationships, we find that of the 82 relationships which may be regarded as falling in this class 78 are positive whereas only 4 are negative in sign.

Averaging the values of the coefficients considered in Table IV, we note that the average for the 133 positive values is +0.3346, whereas that for the 19 negative values is −0.1475. Taking the constants altogether, the average value is +0.2743.

There is, therefore, an overwhelming body of evidence to show that plots, even of the small size and the apparent uniformity of those of the Huntley Station, which yield higher in one year will yield higher persistently throughout a series of years.

It is now desirable to determine whether the same relationships hold when these plots are divided into smaller subplots. It is possible to subdivide a number of the plots into 2 subplots, each one-half the original size. Correlations may be determined for the 92 yields of these half plots in the same manner as for the total yields on the 46 original plots. The results appear in Table V.

The constants are positive throughout. In general, they are statistically significant in comparison with their probable errors. As a matter of fact, only 2 of the 22 constants are less than twice as large as their probable errors. Thus, they indicate a real biological relationship between the productions of the half plots in different years. Those which give a higher yield in one year give a higher yield in another year.

For a smaller number of the crops it is possible to divide the original plots into quarter plots, thus securing 184 subplots to be used as a basis of calculation. The coefficients of correlation between the yields in the several years are shown in Table VI.

	1911, sugar beets.	1912, alfalfa.	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.
1, sugar beets		−0.452 ± 0.079 −5.72	+0.052 ± 0.099 +0.53	+0.011 ± 0.099 +0.11	+0.037 ± 0.099 +0.37
2, alfalfa	−0.452 ± 0.079 −5.72		+.240 ± .094 +2.55	+.187 ± .096 +1.95	+.250 ± .093 +2.69
3, alfalfa I	+.052 ± .099 +.53	+.240 ± .094 +2.55			
Alfalfa II	+.011 ± .099 +.11	+.187 ± .096 +1.95			
Alfalfa I and II	+.037 ± .099 +.37	+.250 ± .093 +2.69			
Alfalfa I	−.018 ± .099 −.18	+.363 ± .086 +4.22	+.898 ± .028 +40.3	+.601 ± .064 +9.39	+.857 ± .027 +31.6
Alfalfa II	−.162 ± .097 −1.67	+.411 ± .083 +4.95	+.394 ± .064 +9.28	+.734 ± .046 +15.0	+.758 ± .034 +20.0
Alfalfa I and II	−.102 ± .098 −1.04	+.420 ± .082 +5.12	+.771 ± .040 +19.3	+.726 ± .047 +15.5	+.815 ± .031 +26.0
Alfalfa III	−.019 ± .099 −.20	+.344 ± .087 +3.92	+.452 ± .079 +5.72	+.624 ± .060 +10.4	+.637 ± .051 +12.5
Alfalfa I to III	−.083 ± .098 −.87	+.420 ± .081 +5.30	+.759 ± .042 +18.0	+.751 ± .043 +17.3	+.881 ± .025 +41.4
Ear corn	+.336 ± .088 +3.82	+.196 ± .098 +1.98	+.034 ± .099 +.34	+.235 ± .094 +2.74	−.109 ± .099 −1.13
Ear corn	+.152 ± .097 +1.57	+.170 ± .097 +1.75	+.647 ± .058 +11.2	+.529 ± .048 +8.0	+.521 ± .051 +9.8
Oat grain	−.024 ± .099 −.24	+.215 ± .095 +2.26	+.358 ± .087 +4.11	−.443 ± .060 +5.54	−.460 ± .057 −6.02
Oat straw	−.116 ± .098 −1.18	+.166 ± .097 +1.71	+.190 ± .090 +1.98	+.208 ± .095 +2.19	+.213 ± .094 +2.42
Total oats	−.098 ± .098 −1.00	+.229 ± .094 +2.44	+.317 ± .089 +3.56	+.372 ± .086 +4.33	+.402 ± .083 +4.87
Silage corn	+.348 ± .087 +4.00	−.071 ± .099 −.72	+.135 ± .097 +1.56	−.451 ± .079 +5.71	−.310 ± .085 +4.02
Barley grain	−.539 ± .070 −7.66	+.527 ± .071 +7.33	+.076 ± .098 +.78	+.203 ± .093 +2.13	+.163 ± .095 +1.68
Barley straw	−.262 ± .092 −2.81	+.341 ± .087 +3.89	−.003 ± .099 −.03	+.025 ± .099 +.26	+.017 ± .098 +.13
Total barley	−.449 ± .079 −5.66	+.483 ± .076 +6.34	+.043 ± .099 +.43	+.131 ± .097 +1.34	+.101 ± .097 +1.05

TABLE V.—*Interannual correlations for yield of 92 half plots in the Huntley uniform cropping experiment*

	1913, alfalfa I.	1913, alfalfa II.	1913, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa I				+0.762±0.030 +25.8	+0.543±0.050 +10.9	+0.715±0.034 +20.8	+0.125±0.069 +1.80	+0.686±0.037 +18.4
1913, alfalfa II				+ .438± .057 +7.71	+ .601± .045 +13.4	+ .580± .047 +12.4	+ .229± .067 +3.43	+ .618± .043 +14.2
1913, alfalfa I and II				+ .707± .035 +20.1	+ .673± .038 +17.5	+ .763± .029 +25.9	+ .208± .067 +3.09	+ .768± .029 +26.6
1914, alfalfa I	+0.762±0.030 +25.8	+0.438±0.057 +7.71	+0.707±0.035 +20.1				+ .228± .067 +3.42	+ .744± .031 +23.7
1914, alfalfa II	+ .543± .050 +10.9	+ .601± .045 +13.4	+ .673± .038 +17.5				+ .112± .069 +1.61	+ .739± .032 +23.2
1914, alfalfa I and II	+ .715± .034 +20.8	+ .580± .047 +12.4	+ .763± .029 +25.9				+ .185± .068 +2.72	+ .820± .023 +35.7
1915, ear corn	+ .125± .069 +1.80	+ .229± .067 +3.43	+ .208± .067 +3.09	+ .228± .067 +3.42	+ .112± .069 +1.61	+ .185± .068 +2.72		+ .329± .063 +5.25
1916, ear corn	+ .686± .037 +18.4	+ .618± .043 +14.2	+ .768± .029 +26 6	+ .744± .031 +23.7	+ .739± .032 +23.2	+ .820± .023 +35.7	+ .329± .063 +5.25	

TABLE VI.—*Interannual correlations for yield of 184 quarter plots in the Huntley uniform cropping experiment*

	1913, alfalfa II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa II		+0.373±0.043 +8.71	+0.373±0.043 +8.71	+0.446±0.040 +11.2	+0.193±0.048 +4.04	+0.512±0.037 +14.0
1914, alfalfa I	+0.373±0.043 +8.71				+ .174±.048 +3.61	+ .642±.029 +22.0
1914, alfalfa II	+ .373±.043 +8.71				+ .109±.049 +2.22	+ .629±.030 +21.0
1914, alfalfa I and II	+ .446±.040 +11.2				+ .158±.048 +3.27	+ .719±.024 +30.0
1915, ear corn	+ .193±.048 +4.04	+ .174±.048 +3.61	+ .109±.049 +2.22	+ .158±.048 +3.27		+ .329±.044 +7.42
1916, ear corn	+ .512±.037 +14.0	+ .642±.029 +22.0	+ .629±.030 +21.0	+ .719±.024 +30.0	+ .329±.044 +7.42	

Unfortunately the number of crops which can be included in Table VI is rather small. The coefficients are positive in sign throughout, and in all cases they are statistically significant in comparison with their probable errors. The individual constants will receive attention in the following discussion.

The fact that the yields are correlated in the different years for whole plots of 0.17 acre, for half plots of 0.085 acre, and for quarter plots of only 0.0425 acre emphasizes the permanence of the substratum differences. We now have to compare the correlations secured for these three divisions. The difference in the actual magitudes of the correlations appear in Table VII. The three entries, when all comparisons are possible, show: (1) the difference between the correlation for whole plots and half plots, (2) the difference between the correlation for whole plots and quarter plots, and (3) the difference between the correlation for half plots and quarter plots.

The signs are positive when the correlations are larger for the larger areas.

The comparisons show that in general the correlations decrease in magnitude as the areas upon which they are based are subdivided. Thus 16 of the 22 comparisons of the correlations deduced from whole plots and from half plots (first entry) show a lower correlation in the half plots as compared with 6 which show higher correlations in the half plots.

TABLE VII.—*Differences in interannual correlations for whole plots, half plots, and quarter plots*

	1913, alfalfa I.	1913, alfalfa II.	1914, alfalfa I and II.	1914, alfalfa I.	1914, alfalfa II.	1914, alfalfa I and II.	1915, ear corn.	1916, ear corn.
1913, alfalfa I				−0.0863	−0.0514	−0.0458	+0.0910	+0.0387
1913, alfalfa II				−.1622	−.1335	−.1462	−.0267	−.1021
				−.2276	−.3615	−.2794	−.0623	−.2081
				−.0653	−.2280	−.1332	−.0355	−.1059
1913, alfalfa I and II				−.1442	−.1044	−.1152	+.0493	−.0230
1914, alfalfa I	−0.0863	−0.1622	−0.1442				−.0385	−.0500
		−.2276					−.0170	−.1578
		−.0653					−.0539	−.1015
1914, alfalfa II	−0.0514	−.1335	−.1044				−.0105	−.0483
		−.3615					−.0609	−.1599
		−.2280					−.0609	−.1165
1914, alfalfa I and II	−0.0458	−.1462	−.1152				−.0039	−.0377
		−.2794					+.0185	−.1388
		−.1332					−.0204	−.1011
1915, ear corn	+0.0910	+.0267	+.0493	+.0388	+.0705	+.0649		−.0492
		+.0623		−.0150	+.0670	+.0485		+.0170
		+.0355		−.0539	−.0039	−.0204		−.0064
1916, ear corn	+0.0387	−.1021	−.0230	−.0500	−.0493	−.0377	−.0492	
		−.2081		−.1578	−.1599	−.1388	+.0170	
		−.1059		−.1018	−.1105	−.1011	−.0064	

Of the 12 comparisons possible between the interannual correlations deduced from whole plots and from quarter plots (second entry), 9 show lower correlations for quarter plots as compared with 3 which show higher correlations for the quarter plots. Finally, all 12 of the correlations deduced from quarter plots are lower than the correlations deduced from half plots.

It appears, therefore, that 0.085 and 0.0425 acre are rather too small to give the highest values of the interannual correlations. On areas of this size other factors than the peculiarities of the plots themselves have too large an influence upon variation of yield to allow the individuality of the plots to express itself fully in its influence upon the yields of successive years.

In support of the conclusion that the lower value of the correlations for half and quarter plots is due to the greater variability of the yields of these plots we note that the coefficients of variation for subplots are without exception larger than those for the plots of the original size. The coefficients of variation are as follows for the years in which the plots were subdivided.

Crop.	Whole plots.	Half plots.	Quarter plots.
1913, alfalfa I	12.52	14.93	.....
1913, alfalfa II	13.60	16.59	21.87
1913, alfalfa I and II	11.11	13.34	.....
1914, alfalfa I	17.94	20.04	23.68
1914, alfalfa II	19.81	21.77	25.87
1914, alfalfa I and II	17.47	18.90	21.88
1915, ear corn	7.29	8.43	9.23
1916, ear corn	13.43	15.88	17.68

It is now desirable to examine the results for the individual crops. In doing this it may be noted that there are two factors to be taken into account. First, there is the possibility of an inherent difference in the plots which is persistent from year to year and is quite independent of the crop grown. Second, it is conceivable that the crop itself may exert an influence upon the soil such that the yields of subsequent crops will be influenced by variations in its growth which are measured in terms of yield.

The first of these factors would influence all correlations between plots—those between the yields of given years and the yields of both preceding and subsequent seasons. The second would influence only correlations with subsequent years.

In a series of only 46 plots it will probably be impossible to distinguish between the influences of these two factors.

We note that the higher yields of beets are followed by lower yields of alfalfa in 1912, but that there is practically no relationship between the yields of sugar beets in 1911 and the yield of other crops on the same

plots from 1913 to 1918. Possible exceptions are ear corn in 1915 and silage corn in 1918, for which the correlations are positive and perhaps statistically significant in comparison with their probable errors. The correlations for yields of sugar beets in 1911 and yields of barley in 1919 are negative in sign and apparently statistically significant in comparison with their probable errors. We have no explanation to offer concerning this apparent relationship. The average value, with regard to sign, of the correlations between the yield of sugar beets and other crops is −0.077.

The correlations between the 9 different cuttings of alfalfa made during 1912 to 1914 and all other yields are generally positive and statistically significant in comparison with their probable errors. The only exceptions are the negative correlation with sugar beets in 1911 which have already been noted and the slight and statistically insignificant correlation for the 1912 yield of alfalfa and the yield of silage corn in 1918.

Since it is quite reasonable to assume that in a crop harvested more than once a year thickness of stand and variation in the size of the individual plants will have a large influence on the yields of the different plots in the same year, the correlations between the different cuttings of the same year as well as those between single cuttings and totals of two or more cuttings in the same year have been omitted from the tables. The correlations between different cuttings in the same year are given in Table VIII.

TABLE VIII.—*Comparison of correlations between different cuttings of alfalfa in the same year*

Cuttings of alfalfa.	Whole plots.	Half plots.	Quarter plots.
1913, first and second cuttings	+0.454±0.079	+0.442±0.057	
1914, first and second cuttings	+ .711± .049	+ .633± .042	+0.558±0.034
1914, first and third cuttings	+ .595± .064		
1914 (first plus second) and third cuttings	+ .653± .057		

We shall now consider the correlations between the yields of alfalfa and between the yields of alfalfa and of other crops on the same plots in different years. The individual constants may be studied in the fundamental table (Table IV). The averages are given in Table IX. This shows that the correlations between different cuttings of alfalfa are on the average larger throughout than those between the yield of alfalfa and the yields of other crops on the same plots.

TABLE IX.—*Comparison of correlations between different yields of alfalfa with correlations between yields of alfalfa and yields of other crops*

Cuttings of alfalfa.	With other cuttings of alfalfa.	With yields of other crops.	Difference.
1912, single cutting	+0.331	+0.171	+0.160
1913, first cutting	+ .611	+ .187	+ .424
1913, second cutting	+ .604	+ .282	+ .322
1913, first and second cuttings	+ .720	+ .274	+ .446
1914, first cutting	+ .666	+ .295	+ .371
1914, second cutting	+ .629	+ .244	+ .385
1914, first and second cuttings	+ .699	+ .290	+ .409
1914, third cutting	+ .524	+ .303	+ .221
1914, first, second, and third cuttings	+ .706	+ .316	+ .390

It is clear, therefore, that either stand or specific adaptation of the individual plots to alfalfa influences to an unusual degree the closeness of correlation between the yields of the plots of alfalfa in the different years.

In the first crop of ear corn (1915) we find higher yields of ear corn in 1916, a negligible difference in the yield of oat grain and straw and total yield in 1917, higher yield of silage corn in 1918, and slightly but not significantly higher yield of barley grain, straw, and total yield in 1919 following higher yield of corn in 1915.

Turning to the constants for ear corn in 1916, we note that higher yields of grain in this year are followed by higher yields of oat straw and grain in 1917 and of silage corn in 1918, and by slightly higher yields of barley grain and straw in 1919.

The average value of the correlation between the yield of ear corn in 1915 and the yield of other crops during the eight years is +0.167, whereas that for ear corn in 1916 and other crops is +0.486. These averages include the correlations for alfalfa, which are, as shown by Table VIII, high for the crop of 1916.

Considering the correlations for oat straw, grain, and total crop on the several plots in 1917 and the yields of silage corn in 1918, we find that higher values of each of these measures of capacity for oat production are on the average followed by slightly, but perhaps not significantly, higher yields of silage corn in 1918 and generally by higher barley yields in 1919.

For the oat yields the average correlations with other crops are: for straw, +0.202; for grain, +0.289; and for total yield, +0.293.

The correlations of the yields of silage corn with the yields of the preceding crops are, with one exception, positive in sign. The average value for the eight years is +0.226.

The averages of the correlations between barley yields and the yields of other crops on the same plots during the eight years of the experiment are +0.141 for grain, +0.086 for straw, and +0.126 for total yield.

Summarizing this discussion of the results for the individual crops, we have the following average values of the correlation coefficients:

1911, sugar beets	−0.077	1915, ear corn	+0.167
1912, total alfalfa	+ .242	1916, ear corn	+ .486
1913, alfalfa I	+ .346	1917, oat straw	+ .202
1913, alfalfa II	+ .403	1917, oat grain	+ .289
1913, alfalfa I and II	+ .441	1917, total oats	+ .293
1914, alfalfa I	+ .401	1918, silage corn	+ .226
1914, alfalfa II	+ .354	1919, barley grain	+ .141
1914, alfalfa I and II	+ .407	1919, barley straw	+ .086
1914, alfalfa III	+ .366	1619, total barley	+ .126
1914, alfalfa I to III	+ .428	General average	+ .274

With the exception of the sugar beets the average correlation for every crop is positive in sign, and in many cases it is of a very material value.

Returning to the averages for the individual crops, we note from Table IX that the lowest correlation for alfalfa, whether with other cuttings of alfalfa or with the yield of other crops, is that for the single cutting of 1912.

It might be suggested that the 1912 yields of alfalfa are less likely to reflect the real producing capacity of the plots than the yields of the later cuttings of this crop, for the reason that the first cutting of alfalfa when sown without a nurse crop is subject to much variation because of slight differences in surface condition of the soil at seeding time and also because of differences in weediness of different plots. Both these conditions would become relatively less important in their effect on crop yield after the first cutting.

Because of its nitrogen-fixing capacity and the resistance to decay of the roots and stubble of alfalfa the correlation between the various yields of this legume and the yields of subsequent crops is of especial interest. Fortunately two crops of ear corn were grown immediately after the alfalfa, which was broken up in the fall of 1914.

A comparison of the correlations of these two series of corn yields with the preceding yields of alfalfa is made in Table X. These coefficients indicate a positive correlation between all the yields of alfalfa and the yields of ear corn in both 1915 and 1916.

Of the 19 correlations determined between the yields of alfalfa for 1912 to 1914 and the yields of ear corn in 1915 only 9 may be looked upon as probably significant in comparison with their probable errors. Of the 19 correlations between the yields of alfalfa in 1912 to 1914 and the yields of ear corn in 1916 only one coefficient—that for the 1912 yield of alfalfa and the 1916 yield of corn—can not be considered as representing a real agronomic relationship between yield of alfalfa and yield of corn.

The constants for 1916 are without exception larger and with two exceptions significantly larger in comparison with their probable errors than those for 1915.

TABLE X.—*Comparison of correlations between yields of alfalfa and yields of the first and of the second subsequent crop of corn*

	Whole plots.			Half plots.			Quarter plots.		
	1915	1916	Difference.	1915	1916	Difference.	1915	1916	Difference.
1912, alfalfa	+0.106±0.098 +1.08	+0.170±0.097 +1.75	+0.064±0.138 +0.46						
1913, alfalfa I	+.034±.099 +.34	+.647±.058 +11.2	+.613±.115 +5.34	+0.125±0.069 +1.80	+0.686±0.037 +18.4	+0.561±0.078 +7.17			
1913, alfalfa II	+.255±.093 +2.74	+.720±.048 +15.0	+.465±.105 +4.45	+.229+.067 +3.43	+.618±.043 +14.2	+0.389±.080 +4.89	+0.193±0.048 +4.04	+0.512±0.037 +14.0	+0.319±0.061 +0.53
1913, alfalfa I and II	+.168±.097 +1.73	+.801±.036 +22.3	+.633±.103 +6.12	+.208±.067 +3.09	+.768±.029 +26.6	+0.560±.073 +7.67			
1914, alfalfa I	+.289±.096 +1.97	+.800±.036 +22.2	+.611±.103 +5.96	+.228±.067 +3.42	+.744±.031 +23.7	+0.516±.074 +6.99	+.174±.048 +3.61	+.642±.029 +22.0	+.468±.056 +8.36
1914, alfalfa II	+.041±.099 +.41	+.789±.038 +20.8	+.748±.106 +7.06	+.112±.069 +1.61	+.739±.032 +23.2	+0.627±.076 +8.25	+.109+.049 +2.22	+.629±.030 +21.0	+.520±.057 +9.06
1914, alfalfa I and II	+.120±.098 +1.22	+.858±.026 +33.0	+.738±.101 +7.29	+.185±.068 +2.72	+.820±.023 +35.7	+0.635±.072 +8.86	+.158±.048 +3.27	+.729±.024 +30.0	+.561±.054 +10.5
1914, alfalfa III	+.329±.088 +3.71	+.710±.049 +14.4	+.381±.101 +3.78						
1914, alfalfa I to III	+.191±.095 +2.00	+.880±.022 +39.4	+.689±.098 +7.07						
Average	.1592	.7083	.5491	.1811	.7291	.5480	.1585	.6255	.4670

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The average value for the nine pairs of correlations deduced from the yields of whole plots is +0.159 for alfalfa and corn yield in 1915 but +0.708 for alfalfa and corn yield in 1916. For the six pairs of correlations which may be deduced for half plots the average of the coefficients for the various yields of alfalfa in 1913 and 1914 and the yield of ear corn in 1915 is +0.181, whereas the average correlation of the same yields of alfalfa with corn one year later is +0.729. Finally, in the four cases in which it was possible to calculate correlations between alfalfa and corn yields on the basis of data for quarter plots the average for the correlations with ear corn in 1915 is +0.159, whereas the constants showing the relationship between the yield of alfalfa in 1913 and 1914 and ear corn in 1916 give an average of +0.626.

This more intimate relationship between the yields of alfalfa and the second crop of ear corn does not necessarily mean that the corn crop of 1916 was larger than that of 1915 but merely that the variations in the individual plot yields in 1916 are more dependent than those of 1915 upon the yields of alfalfa during 1912 to 1914. As a matter of fact the average yield in 1915 was 522.6 pound per plot, while in 1916 it was 396.2 pounds per plot. The greater yield in 1915 may have been, and probably was, due to factors other than soil conditions as such.

It is of interest in this connection to turn back to the table of coefficients of variation of yield (p. 347) and to note that for whole plots, half plots, and quarter plots the coefficients of variation of plot yield are distinctly lower in 1915 than in 1916. This result is quite in line with what one would expect if the fixed nitrogen of the varying growths of alfalfa were not yet fully available in 1915.

There is also another possible explanation for the lower correlation between the alfalfa yields and the yields of corn in 1915. It is always a difficult matter on the heavy soils at Huntley to break up alfalfa sod and to get the soil into good tilth for the succeeding crop. It may be that some of the plots in this field include heavier soil which ordinarily gives good yields but which was harder to get into good condition in time for the 1915 corn crop. If this were the case, these differences in tilth might have been smoothed out by the season's cultivation so as not to be expressed in the 1916 crop yields.

Some light may be thrown upon the problem of the residual influence of alfalfa in the following manner.

If the correlations between the plot yields of later crops be in a large degree determined by differences in fertility referable to differences in stand and yield of the preceding alfalfa crops, one might expect a closer correlation between the yields of ear corn in 1916 and oats in 1917 than between ear corn in 1916 and ear corn in 1915, since, as is shown above, variations in the alfalfa yields have little influence until 1916. This will be true, provided there be a residual influence of the variations in the yields of alfalfa such that these variations in fertility due to varia-

tions in yield from 1912 to 1914 inclusive will influence not merely the yield of corn in 1916 but the yield of oats in 1917, etc. The correlations between corn yields in 1915 and corn yields in 1916 and the yields of subsequent crops are shown side by side in Table XI.

TABLE XI.—*Comparison of correlations of the yields of ear corn in 1915 and in 1916 with the yields of subsequent crops*

	Corr. 1915.	Corn, 1916.	Difference.
1917.			
Oat grain	−0.025±0.099	+0.497±0.075	+0.522±0.124
Oat straw	+.112±.098	+.220±.095	+.108±.136
Total yield	+.072±.099	+.407±.083	+.335±.129
1918.			
Silage corn	+.459±.078	+.439±.080	−.020±.112
1919.			
Barley grain	+.042±.099	+.104±.098	+.062±.139
Barley straw	+.184±.096	+.144±.097	−.040±.136
Total yield	+.119±.098	+.135±.097	+.016±.138

These comparisons show that the yields of oats in 1917 are much more closely correlated with the yields of corn in 1916 than with the yields of ear corn in 1915. No such relationship is apparent in the correlations for silage corn in 1918 or for barley in 1919. The after effect of the alfalfa crops of 1912 to 1914 is, therefore, apparently largely limited to an influence on the yield of oats in 1917.

Turning from this indirect to a more direct method of comparison, we have determined the averages of the correlations between the several individual cuttings of alfalfa and the yields of the single antecedent and of the five subsequent crops. The results are given in Table XII.

TABLE XII.—*Averages of the correlations between the cuttings of alfalfa in 1912 to 1914 and the antecedent and succeeding crops*

Crop correlated with alfalfa.	Grain.	Straw.	Total yield.
Sugar beets, 1911			−0.082
Ear corn, 1915	+0.159		
Ear corn, 1916	+.708		
Oats, 1917	+.437	+0.210	+.371
Silage corn, 1918			+.279
Barley, 1919	+.234	+.113	+.195

There should be no correlation between the yield of sugar beets and alfalfa except that due to the initial heterogencity of the field. The

insignificant negative correlation observed may be due to some peculiarity of the crop. The comparison of the correlation for the 1915 and 1916 corn crops has already been made (Table XI). Inspection of the averages in Table XII shows that on whatever character they are based the correlations decrease from the maximum relationship observed in 1916 to the lowest values in 1919.

Whether the residual influence of alfalfa *per se* has any influence on the 1919 or later crops can only be determined by further experimentation in which the interannual correlations can be deduced from the yields of plots upon which alfalfa has not been grown.

III.—DISCUSSION AND RECAPITULATION

The purpose of this paper has been to present the results of a new method of attack upon the problems of (*a*) the permanency of the differences which are found in the plots of an experimental field, and of (*b*) the influence of variations in the yields of certain crops in the rotation upon the yields of subsequent crops.

The data upon which the studies were primarily based comprise the yields of 46 plots—subdivided in several cases into half plots and quarter plots—each of 0.17 acre in area at the Huntley (Mont.) Field Station of the Office of Western Irrigation Agriculture for the nine years between 1911 and 1919, inclusive.

The uniform cropping experiment, involving sugar beets, alfalfa, corn, oats, and barley, was initiated merely to determine the variation in the yields of plots of a given size when homogeneously planted and uniformly treated. The experimental procedure was, therefore, determined in advance and was wholly independent of the statistical analysis. This is in certain regards fortunate. It frees the data absolutely from any suspicion of an influence of preconceptions or of personal equation on the biometric results. On the other hand, it is quite possible after the statistical analyses have been made to recognize ways in which the experiments could have been improved and made to yield more valuable results. This is, however, a feature of research in general. The discovery of inadequacies in a first set of experiments makes possible their elimination in subsequent work. The most unfortunate defect in the data was that the harvesting and weighing could not be done by half and quarter plots in 1917, 1918, and 1919, but this curtailment could not be avoided under existing conditions.

The results of a previous study (*3*) have shown that fields selected for plot tests of all kinds are practically without exception heterogeneous to a degree that influences profoundly the yields of the crops grown upon them. It was there pointed out that the correlation between the yields of adjacent plots might either be due to initial physical and chemical differences in the soil or be referable to the influence of previous crops upon the composition, texture, or tilth of the soil.

The first purpose of the present study has been to determine whether such differences in fields selected for their apparent uniformity by skilled agronomists are of a purely transitory nature or whether they are of a relatively permanent character.

This problem can be solved by determining whether in such series of uniformly treated plots the yields of the same plots in different years are correlated.

The results of the present study show that of the 152 correlations between the yields of the plots in different years, 133 are positive as compared with 19 which are negative in sign. The average value of the positive correlations is + 0.335, whereas the average of the negative constants is − 0.148. The general average is + 0.274. With the exception of the 1911 crop of sugar beets the correlation between the yields of each individual crop and the yields on the same plots in the eight other years of the experiment are on the average positive.

The data available for half and quarter plots fully substantiate the results for whole plots.

The results show conclusively, therefore, that plots, even of the small size and apparent uniformity of those at the Huntley Station, are characterized by differences which may persist throughout a period of years. Thus, in general, plots which produce more in one year will produce more in another year.

This is, of course, a well-recognized principle for large tracts. Its validity for small plots has apparently not been recognized heretofore. It is probably not a principle of universal applicability, because of the fact that meteorological as well as soil conditions play a large part in determining yield. It is quite probable that certain soil characteristics would result in maximum yields with one set of meteorological conditions but in minimum yields with another complex of aereal conditions.

The determination of the proximate factors to which these correlations are due presents a problem of considerable difficulty. Unfortunately (for this phase of the problem only) alfalfa was introduced early in the rotation and occupied the ground for three of the nine years covered by the experiment. It seems quite possible that the correlations between certain of the yields is due in part to the variation in nitrogen content of the soil referable to the variation in thickness of stand and strength of growth of the alfalfa crops.

The results show that there is but little correlation between the alfalfa yields of 1912 to 1914 and the ear corn yields of 1915, whereas the correlations for ear corn in 1916 are high. Thus the influence of alfalfa upon the yield of a subsequent crop is not fully evident until the second year after it is turned under.

There is a definitely demonstrable residual influence of the variation of alfalfa yields upon the yields of subsequent crops. The influence of

the alfalfa upon the yield of subsequent crops decreases with the lapse of time from the maximum correlation found for ear corn in 1916. The residual influence of the alfalfa is clearly marked in the oat crop of 1917 and may still be evident in the silage corn and barley crops of 1918 and 1919.

In view of the early introduction of alfalfa into the rotation, it is impossible to determine whether the correlations between yields other than those for alfalfa are due to the variation from plot to plot of the amount of nitrogen fixed by the alfalfa or whether it is to a considerable extent due to the original heterogeneity of the plots. This and other problems which will suggest themselves to the reader can be solved only by the analysis of further experimental data. The illustrations of the present paper are sufficient to show the value of the application of the interannual correlation method to agronomic problems.

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SOME CHANGES IN FLORIDA GRAPEFRUIT IN STORAGE[1]

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INTRODUCTION

Zoller (*11*),[3] in his paper on the constituents of the grapefruit (*Citrus decumana*), has pointed out that very little attention has been paid to the chemical constituents of this important fruit. This statement might also be made concerning the physiology of the fruit and the changes which go on in it after it is removed from the tree and held at storage temperatures. Some analyses of grapefruit have been made, however, by various investigators.

Chace, Tolman, and Munson (*4*) in their work on tropical fruits analyzed several different varieties of grapefruit. Rose (*8*) and others connected with the Florida Agricultural Experiment Station have made many analyses of citrus fruits in working out a basis for the regulation of the shipping of them. These last-mentioned analyses were for the most part determinations of the acid and sugar content of the pulp or juice and of the soluble solids present in the juice.

Collison (*5*) determined the acids and sugars in the juice of several varieties of grapefruit picked at various times during the season. He found, in general, that there was a decrease in acidity and an increase in sugar content as the season advanced and that after the fruit matured the sucrose was gradually broken down to reducing sugars. The fruits were analyzed shortly after removal from the tree.

Shamel (*9*) quotes a number of analyses of Florida and California grapefruit by E. M. Chace. Zoller (*11*) found that the acid of the pulp decreased during storage and records a marked increase in sugars after the fruit is removed from the tree. He found also that the content of the glucoside naringin, which is the bitter principle of grapefruit, was less in the peel after storage. This writer apparently used only a small number of fruits in his storage experiments, the work being for the most part a chemical study of the various constituents of the fruit.

Chace and Church (*3*) recently made a chemical study of some different types of grapefruit grown in California and Arizona. They determined

[1] This paper gives the result of a portion of the work carried on under the project "Factors Affecting the Storage Life of Fruits."

[2] The writers' thanks are due Mr. L. B. Scott, formerly Pomologist, Office of Horticultural and Pomological Investigations, for advice and helpful criticism while this work was in progress.

[3] Reference is made by number (italic) to "Literature cited," p. 372–373.

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the acid-solids ratio of grapefruit picked at intervals throughout the season from a number of localities. Some little work was also done on the effect of cold storage and storage in lemon curing rooms on the acid-solids ratio of the juice as compared to that of similar fruit direct from the tree. The data given seem to show that there is an increase in the acid-solids ratio during storage.

While other investigations have been carried out on certain chemical phases of the composition of grapefruit, the articles mentioned above are apparently all that are of interest in connection with the present work.

It is evident from the brief review of the literature here presented that the longer the fruit is held on the tree the lower the acid content. The acid content also apparently decreases during storage. The sugar content increases in fruit on the tree as the season advances, and some evidence is brought out that it increases during storage.

The present investigation was taken up to determine the effect of storage at various temperatures on the fruit and particularly on the sugar and acid content of the pulp, since these substances make up the major portion of the dry matter of the pulp or interior of the fruit, without the seeds.

METHODS OF EXPERIMENTATION

The fruit used in these experiments was from single trees of two named varieties, Silver Cluster and Davis, and "common Florida."[1] Most of the work was done with the two varieties last mentioned, the fruit of these varieties all being from three trees, one Davis and two "common Florida."[2]

The fruit from each tree was packed separately and was shipped to Washington, where the storage experiments were carried out. The first season's experiments, those of 1917–18, were preliminary, and only Silver Cluster fruit was used. All the fruit was obtained from one tree. It was shipped to Washington, where part of it was stored at 86° F. and the rest placed in a commercial cold storage at 32°. In the experiments with this fruit, the juice alone was analyzed, though the comparative percentage of peel and pulp was determined in some cases. The method followed was to peel the fruit, grind the pulp, and press out the juice through thin muslin. The acid-solids ratio was determined according to the usual method (*8*), and samples were, in most cases, made for sugar determinations. The samples for sugar determinations were pipetted into 250-cc. volumetric flasks, cleared with neutral lead acetate, made up to volume, filtered, and the excess lead removed with sodium oxalate. The reducing substances in this solution were determined. For total

[1] The writers are indebted to Mr. W. J. Krome, of the Medora Grove, Homestead, Fla., for his kindness in picking, packing, and shipping the fruit from these three trees at various times during the season, and to Mr. F. S. Poole, of Lake Alfred, for the Silver Cluster fruit used in the first season's work.

[2] "Common Florida" is the name applied in Florida to fruit of seedling grapefruit trees or trees budded from seedlings to which no distinctive varietal name has been applied. The term, therefore, may include fruit which represents a rather wide range in some of its characteristics.

sugars a 50-cc. aliquot was pipetted into a 100-cc. volumetric flask, the sucrose inverted by adding 5-cc. of concentrated hydrochloric acid and allowing it to stand overnight at room temperature. This solution was made up to volume, neutralized, and the reducing substance in it was determined.

Matthews's modification of Bertrand's method (*7, p. 994*) was followed in the determination of the sugars. The sugars were calculated as dextrose according to Munson and Walker's tables (*10*).

PRELIMINARY EXPERIMENTS, 1917–18

Table I shows the results obtained from several experiments in which fruit was placed in the incubator maintained at 86° F. In these experiments a sample consisting of six or more fruits was analyzed when the fruits were placed in the incubator, and analyses were made at the dates indicated in the first column. In experiment 2 fruit of the same lot as that used in experiment 1, which had been kept in cold storage since November 28, was placed in the incubator on January 6. The analyses on this latter date give data as to the effect of storage at 32° on the acid and sugar content of the fruit. The change in the acid-solids ratio of this fruit maintained at 86° for 15 and 28 days is shown in the table.

TABLE I.—*Changes in the composition of Silver Cluster grapefruit during storage at 86° F. as indicated by the change in acidity and sugar content of juice*

EXPERIMENT 1

Date sampled.	Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Sugar as dextrose.		
				Reducing.	Sucrose.	Total.
	Per cent.			*Per cent.*	*Per cent.*	*Per cent.*
Nov. 28, 1917	1.16	9.15	7.9 : 1	2.95	2.76	5.71
Dec. 8, 1917	1.14	9.81	8.6 : 1	2.98	2.79	5.77
				3.03	2.59	5.62
Jan. 5, 1918	1.09	10.97	10.06 : 1	3.49	2.28	5.77

EXPERIMENT 2; FRUIT PLACED IN INCUBATOR

Jan. 6, 1918	1.14	8.95	7.8 : 1	2.90	2.68	5.58
				2.87	2.59	5.46
Jan. 21, 1918	.83	9.83	11.8 : 1	.....	.....	.....
Feb. 3, 1918	.76	9.29	12.2 : 1	.....	.....	.....

From Table I it is evident that there is a decrease in acidity when the fruit is stored at warm temperatures, while there is little, if any, decrease in the total sugar content of the juice. The reducing sugar is increased somewhat, but there is a corresponding decrease in the cane

sugar. The acid-solids ratio increases markedly in storage at 86° F., but there is no evidence of change at 32° in 38 days.

Some idea of the shrinkage in grapefruit and the change in acid-solids ratio was obtained in another experiment in which eight grapefruits which had been stored for 13 days were removed from storage, weighed, four of them peeled, and the percentages of peel and pulp determined. Acid, sugar, and soluble solids were determined in the juice of these four fruits. The other four fruits were placed in the incubator at 86° F. and allowed to remain 12 days. They were then removed, weighed, and the percentage of shrinkage, the percentage of peel, and the acid and soluble solids determined according to the usual method. The data obtained from these determinations are shown in Table II.

TABLE II.—*Acids, soluble solids, acid-solids ratio, shrinkage, and peel in single Silver Cluster grapefruit*

Fruit No.	When placed in storage.				Fruit No.	After 12 days' storage at 86° F.				
	Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Peel.		Acid as citric.	Soluble solids (Brix).	Acid-solids ratio.	Shrinkage of fruit.	Peel.
	Per cent.			*Per cent.*		*Per cent.*			*Per cent.*	*Per cent.*
5.....	1.07	9.75	9.1 : 1	26	1.....	0.95	11.67	12.27 : 1	24	23
6.....	1.17	9.15	7.7 : 1	28	2.....	.93	11.61	12.49 : 1	32	18
7.....	1.12	9.09	8.1 : 1	27	3.....	1.00	11.07	11.07 : 1	26	21
8.....	1.14	10.35	9.1 : 1	27	4.....	1.02	11.61	11.37 : 1	35	18

The data in Table II show that the acidity decreases markedly and that the acid-solids ratio is much higher after storage for 12 days at 86° F. Much of this apparent increase in soluble solids is probably due to a concentration of the juice by the loss of water from the fruit. Inasmuch as the average shrinkage of the fruit is 29 per cent, while the average percentage of peel dropped from 27 to 20 per cent, obviously much of the water given off comes from the pulp.

EXPERIMENTS IN 1918–19

In the second season's work, Davis and "common Florida" grapefruits were obtained from Mr. W. J. Krome, Homestead, Fla. The entire crop from three trees was used in the storage experiments, one picking being made from the Davis tree and two from the "common Florida" trees. The fruit was shipped to Washington by express and stored at the cold-storage plant at Arlington Farm. Cold-storage temperatures of 32°, 36°, and 40° F. were used as well as common storage at a mean temperature of about 50°, probably fluctuating 5° above and below that temperature, and two warm storage temperatures which were about 70° and 86°, respectively. In most cases the fruit was weighed when placed in storage so that the skrinkage could be determined.

The structure of citrus fruit makes the study of the physiological changes taking place in it rather difficult. Considering the peel, pulp, and seeds of the fruit, there are then three structures which have very different water contents and water-holding powers. It is impossible to grind the entire fruit and weigh out comparable samples. It would be impossible to slice the fruit and expect the various slices to be comparable because of loss of juice from the pulp in slicing and the fact that the seeds are not necessarily evenly distributed. If fruits are sliced and the seeds removed, the operation is liable to be attended with a considerable loss of juice. After a number of experiments, the following method of sampling was decided upon. After the fruit was weighed it was peeled by making two cuts through the skin completely around the fruit, the cuts crossing each other at right angles at the stem and blossom ends. The peel was removed, and the thickness of each quarter was measured midway along the side by means of callipers. Such portions of the rag as adhered to the fruit were removed, and the fruit was weighed again. The percentage of peel was calculated from the weights before and after peeling. The fruit was divided into segments, and the seeds were removed, care being taken that no appreciable amount of juice was lost. Duplicate samples were made from segments from opposite sides of the fruits. One segment from each of the 10 fruits was used for each sample. While this method is not the most accurate, the results of analyses of duplicate samples indicate that it is sufficiently accurate for the work. It must always be taken into account that no two grapefruits have precisely the same chemical composition and that while in this work lots of 10 fruits were commonly used in each set of analyses, some variation will occur between any two lots no matter how carefully the fruits are selected.

In preparing the samples for analysis, the samples for sugar determinations were placed in beakers and covered with 95 per cent alcohol. A few drops of ammonia were added to neutralize the acidity, and the sample was brought to a boil. It was then transferred to extraction thimbles, the alcohol extract was separated at the same time by filtration, and the residue was subjected to continuous extraction for about 14 hours with alcohol in a soxhlet apparatus. The extract was added to the filtrate, the whole was made up to 1,000 cc. in a volumetric flask, and two 50-cc. aliquots were pipetted off for analysis. Sugar determinations were made according to the method already described.

For the acid determinations, the pulp was brought to a boil in water and was placed in liter volumetric flasks under toluol and allowed to stand with frequent shakings for about 10 days. It was then strained through linen, and two aliquots were titrated against sodium hydroxid, using phenolphthalein as an indicator. The dry-weight determinations were made by covering the samples with 95 per cent alcohol, driving off

the alcohol on a steam bath, and drying in a vacuum oven until there was no appreciable loss in weight between successive weighings. The results of the sugar, acid, and dry-matter determinations were calculated to percentage of wet weight of pulp. The percentage of peel was determined by weighing before and after peeling.

COLD AND COMMON STORAGE

As mentioned earlier in this paper, two pickings were made from the two "common Florida" trees, while all the fruit from the Davis tree was picked at the same time as the first lots from the other two trees. The first fruits were harvested October 31 and, as the cold-storage rooms were not yet completed, were allowed to remain in common storage at mean temperature of about 55° F. until November 21, when they were sampled. The fruit was then placed in the various storage chambers. The results of the analyses of the fruit held at 32°, 36°, 40° at various times during the storage season appear in Tables III and IV. The time in days after they were first sampled, when they were placed at the various storage temperatures, is given in the first columns, and the percentage of acid, sugar, dry matter, and the shrinkage of peel and percentage and thickness of peel appear in order. The second lots of fruit from the two "common Florida" trees were picked November 26, and the fruit was placed in the three cold-storage chambers December 4. Some of this picking was also placed in common storage, and the results of analyses of the fruit held in this type of storage are included with the data from the three cold-storage temperatures in Table III.

An inspection of Tables III and IV shows that there is a general decrease in tritratable acids during storage. This decrease would be more marked if it were possible to take into account the shrinkage of the fruit in storage. The actual decrease in acid would be somewhat more than that shown in the table.

In comparing the acid content of the fruit held at the three different cold-storage temperatures, 32°, 36°, and 40° F., it is evident that there is no constant difference in the rate at which the acid decreased. In most cases, however, at comparable samplings the fruit from the 40° storage is somewhat lower in acid content. This is especially noticeable in the Davis fruit (Table IV), where the fruit from the 32° storage is in all four samplings higher in acid content than that fruit from the other two cold storages.

The "common Florida" fruit in common storage was in general lower in acid than comparable lots in cold storage, with the exception of the second sampling which was made 42 days after the fruit was placed in storage. There was undoubtedly a greater shrinkage in the fruit in common storage, as was evidenced by the fact that the peel was thinner and the percentage of dry matter increased in the latter part of the season.

TABLE III.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit at various times during storage season*

TREE I, FIRST PICK; PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	1.02 1.05	2.16	2.17	4.33	8.20 8.14	.....	24.8	.....
After 60 days	.93 .94	2.71	2.35	5.06	8.06	.....	24.2	6.13
After 102 days	.97 .94	2.72	2.15	4.87	8.26 8.01	4.7	23.4	5.64
After 178 days	.89 .89	2.84	2.14	5.06	8.40 8.53	8.0	24.5	4.50
STORED AT 36° F.								
After 102 days	0.94 .97	2.35	2.27	4.62	8.51 8.14	3.8	24.4	6.22
After 178 days	.88 .90	2.86	2.04	4.90	8.6	5.9	22.7	4.30
STORED AT 40° F.								
After 60 days	0.98 .97	2.76	2.20	4.96	8.9	2.2	24.0	5.31
After 102 days	.91 .97	2.49	2.30	4.79	8.42 8.54	4.1	21.2	5.30
After 178 days	.87	2.69	2.16	4.85	8.13 8.04	5.5	22.1	4.00

TREE I, SECOND PICK; PLACED IN STORAGE DEC. 7, 1918

STORED AT 32° F.

When placed in storage	1.28 1.23	2.61	2.82	5.43	8.90 8.78	.....	.....	5.56
After 61 days	1.02	2.84	2.49	5.33	8.79	3.1	24.8	5.87
After 109 days	1.07	2.91	2.33	5.14	9.20 9.16	4.5	23.0	5.40
After 165 days	.89 .90	3.26	2.65	5.91	9.48 9.77	8.5	24.5	4.60
STORED AT 36° F.								
After 109 days	1.00 .98	2.85	2.66	5.51	9.22	3.6	23.0	5.90
After 157 days	1.00 .98	3.39	2.76	6.15	9.44 9.91	5.8	23.0	4.00
STORED AT 40° F.								
After 61 days	1.06 .98	2.58	2.43	5.01	9.00	2.6	22.7	5.57
After 167 days	1.01 .96	3.02	2.66	5.67	9.04 9.44	6.6	19.6	3.90
COMMON STORAGE								
After 42 days	1.11 1.22	3.24	2.45	5.69	8.90 9.02	.....	24.5	5.33
After 121 days	1.02 1.09	3.29	2.45	5.74	9.20 9.16	.....	24.4	4.60
After 179 days	.92 .77	3.00	3.17	6.17	10.00	.....	24.6	4.60

TABLE III.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit at various times during storage season*—Con.

TREE 2, SECOND PICK; PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	1.03 1.11	2.44	2.32	4.76	8.36 8.20	.....	24.6	6.30
After 61 days	.92 .92	2.63	2.43	5.06	8.60 8.22	.....	24	5.98
After 104 days	.98 .94	2.76	2.44	5.20	8.86 8.97	5.7	23.9	6.20
After 179 days	.90 .89	2.89	2.40	5.29	8.70 8.60	6.5	26.3	4.30

STORED AT 36° F.

After 104 days	.97 .98	2.68	2.47	5.15	8.70 8.68	5.7	24.2	6.20
After 153 days	.89 .91	3.03	1.78	4.81	8.77	5.7	24.2	4.20

STORED AT 40° F.

After 61 days	.96	2.90	2.44	5.34	8.75	2.6	26.4	6.73
After 106 days	.92 .90	2.87	2.08	4.95	8.88 8.67	7.2	25.6	5.30
After 153 days	.89 .91	3.03	2.15	5.18	8.70 8.60	6.5	28.3	4.30

TREE 2, SECOND PICK; PLACED IN STORAGE DEC. 7, 1918

STORED AT 32° F.

When placed in storage	1.12 1.12	2.77	2.43	5.20	8.96 9.12	.....	24.8	6.08
After 62 days	.....	3.38	2.94	6.32	9.4 9.49	2.9	22.6	6.09
After 109 days	.94 .93	3.07	2.86	5.93	.....	5.1	24.2	5.90
After 169 days	.97 .94	3.06	2.90	5.96	9.12 9.84	7.8	19	5.00

STORED AT 36° F.

After 109 days	1.62 .99	2.99	2.70	5.69	9.59 9.28	4.8	.....	.....
After 157 days	.96	3.32	2.59	5.91	9.80 9.89	5.6	24.8	5.00

STORED AT 40° F.

After 62 days	0.94	.....	.....	.....	9.7 9.6	2.5	24.5	6.09
After 1.5 days	.93 .91	3.03	2.36	5.39	9.44 9.72	6.5	24.2	4.60

COMMON STORAGE

After 42 days	1.06 1.07	3.55	2.23	5.78	9.43	.....	24.3	.....
After 111 days	.94 .91	3.23	2.12	5.35	10.10 9.77	.....	22.7	4.74
After 179 days	.83 .80	3.56	2.28	5.84	9.84	.....	20.4	3.70

TABLE IV.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of Davis grapefruit at various times during storage season*

PLACED IN STORAGE NOV. 21, 1918

STORED AT 32° F.

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	0.93 .96	2.69	1.66	4.35	7.94 8.10	.....	23.7	5.10
	.91				8.41			
After 58 days	.97	3.06	2.29	5.35	8	3.5	23.1	4.73
After 88 days	.86	3.05	2.09	5.14	8.37	3.9	23.4	5.16
After 118 days	.83 .83	3.25	2.02	5.27	8.73 8.09	3.3	25	4.8
After 140 days	.84 .84	3.09	2.03	5.22	8.91	5.5	26.5	5.6
STORED AT 36° F.								
After 58 days	0.87	3.11	2.23	5.34	8.43 8.42	2.6	23.5	4.72
After 88 days	.79 .76	3.10	2.14	5.24	8.39 8.25	5.3	22.1	4.56
After 118 days	.80 .75	3.00	2.10	5.10	8.27 8.69	6	22.1	4.7
After 139 days	.78 .80	3.16	2.73	5.99	8.02 8.26	8.7	22.5	4
STORED AT 40° F.								
After 58 days	0.85	3.62	2.04	5.66	8.53	1.2	23.3	4.7
After 88 days	.79	3.05	2.10	5.15	8.5 8.41	7.6	19.9	4.02
After 118 days	.77 .73	3.14	2.09	5.23	8.43 8.13	8.1	18.9	3.9
After 139 days	.73 .75	3.18	2.14	5.31	8.47	7.5	19.2	3.9

A comparison of the acid content of the fruit from the two different pickings, when placed in storage, showed that the fruit picked last has a somewhat higher acid content, probably because the fruit of the first picking stood in common storage 22 days before the first analyses.

The sugar content of stored fruit is in rather striking contrast to the acid content. With few exceptions, the percentage of total sugar is higher in the stored fruit than in the samples analyzed when the fruit was placed in storage. In some cases, as in the Davis fruit (Table IV), which had been stored 139 days at 36° F., the sugar content is more than 30 per cent higher than in the analyses made when the fruit was placed in storage. The difference is as marked in other cases. In general, however, the increase in total sugar content is more apparent than real and is probably due to the loss of water from the fruit. The shrinkage of the fruit is in many cases sufficient to account for the apparent increase in sugar content. It is, however, undoubedly true that there is no appreciable diminution of the sugar content during storage at the four temperatures here considered.

The sucrose content, when calculated as percentage of pulp, remains about the same during storage. Apparently the breaking down of the sucrose just about keeps pace with the shrinkage of the fruit. This

increase in total sugars, then, as the storage season advances, is due to an increase in free-reducing substances.

The dry-matter determinations are not particularly conclusive in the analyses here shown. A careful inspection of the data obtained from the 17 storage experiments shown in Tables III and IV indicates that there is, in general, an increase in dry matter. This is probably due to the loss of water from the fruit as well as to losses from respiratory activities, both of which are included in shrinkage.

The shrinkage increases with the length of time the fruit remains in storage and is in general around 5 per cent for the first 100 days in cold storage. Only in two cases is it more than 8 per cent for the entire time the fruit was stored. There is no marked difference in shrinkage in the three temperatures. That the shrinkage is from the pulp as well as the peel is shown by the fact that the decrease in the percentage of peel is not sufficient to account for the loss in weight.

In general, the peel is from 19 to 25 per cent of the fruit used in these experiments, and there is no wide variation between the two varieties. The decrease in thickness of the peel during storage is about 30 per cent, due, probably for the most part, to loss of water.

WARM STORAGE

As mentioned in the earlier part of this paper, in addition to the three cold-storage and one common-storage temperatures, grapefruits were placed in two warm storages at temperatures of about 70° and 86° F. Some lots of fruit were stored in boxes and others in lard cans with tight-fitting lids, the lids being removed from the cans occasionally for a short time to aerate the fruit. The storage season for this fruit was, of course, not so long as for that stored in the cold- or common-storage temperatures. The results of analyses of fruit stored at 70° are shown in Table V, while data obtained from the 86° storage are given in Table VI.

TABLE V.—*Percentage of sugars, acids, dry matter, peel, and thickness of peel of "common Florida" grapefruit stored at about 70° F. in ventilated and unventilated packages*

TREE 1, FIRST PICK

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.			
							Mm.
When placed in storage	1.02 1.05	2.16	2.17	4.33	.....	24.8	.....
After 61 days, unventilated	.98 1.03	2.17	1.82	4.69	8.68 8.47	.....	5.01

TREE 2, FIRST PICK

When placed in storage	1.03 1.11	2.44	2.32	4.76	8.36 8.20	24.6	6.00
After 50 days, unventilated	1.02 1.01	2.96	1.95	4.91	8.9 8.45	24.5	6.13
After 50 days, ventilated	.94 .93	2.98	2.40	5.38	9.90	17.12	3.03

TABLE VI.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of grapefruit stored at about 86° F. in ventilated and unventilated packages*

TREE 1, "COMMON FLORIDA," FIRST PICK

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
When placed in storage	1.02 1.05	2.16	2.17	4.33	8.20 8.14	.....	24.8	.....
After 30 days, unventilated	1.04 1.12	2.77	2.01	4.78	9.12 9.30	.....	24.6	5.77
After 30 days, ventilated	1.26 1.19	2.88	2.09	4.97	9.01 9.33	.....	20	3.12

TREE 1, "COMMON FLORIDA," SECOND PICK

When placed in storage	1.28 1.23	2.61	2.82	5.43	8.90 8.78	.....	.....	5.56
After 60 days, ventilated	1.25 1.30	.....	.....	.....	10.44	26.9	12.5	2.41
After 86 days, ventilated	1.16 1.18	3.98	2.02	6.00	12.09	34.6	11.5	2.08

TREE 2, "COMMON FLORIDA," FIRST PICK

When placed in storage	1.03 1.11	2.44	2.32	4.76	8.96 9.12	.....	24.6	.....
After 30 days, unventilated	1.14 1.12	3.28	2.02	5.30	.....	.....	24.3	5.95

TREE 2, "COMMON FLORIDA," SECOND PICK

When placed in storage	1.12 1.12	2.77	2.43	5.20	8.96 9.12	.....	24.8	6.08
After 61 days, ventilated	.96 1.15	3.95	1.99	5.94	10.10 9.75	25.3	13.8	2.42
After 86 days, ventilated	1.26 1.20	4.32	2.23	6.55	12.23	34.7	11.4	2.64

DAVIS

When placed in storage	0.93 .96	2.69	1.66	4.35	7.94 8.10	.....	23.7	.....
After 19 days, unventilated	.90 .96	2.88	2.32	5.20	8.33 8.21	.....	23.7	.....
After 24 days, ventilated	.94	2.78	2.29	5.07	9.76	.....	24.7	.....

In an inspection of the tables it may be seen that in general there is very little, if any, decrease in titratable acids in the fruit stored in cans, that is, in unventilated packages, at either of the two temperatures. In some cases there is an apparent increase, as in tree 1 of "common Florida," first pick (Table VI), which had been stored 30 days at 86° F. and again in tree 2 of the same variety, pick, temperature, and length of storage period. The increase in total sugar content is more, comparatively, in both these cases than is the increase in acid. In all other cases the fruit in the unventilated package has an acid content about

the same as when placed in storage and an increased sugar content. There is some loss of water from the fruit even in the cans which are closed most of the time, and it is possible that the acid decreases, the decrease in most cases being as rapid as the shrinkage of the fruit. It is, of coulse, always possible that at these high temperatures and under the low oxygen pressures some acid is formed in respiration.

With the stored fruit in ventilated packages the analyses made after 24 or 30 days, as shown in Table VI, gave an acid content as high as or higher than when the fruit was placed in storage. At the longer storage periods in both temperatures the acid content was usually lower than at the beginning of the storage period. In every case there was a marked increase in sugar content, as calculated to wet weight of pulp. This increase was greater where the fruit had been in storage more than 30 days.

While no exact data are at hand, it seems probable that the increase in acid is due, for the most part, to loss of water from the fruit. Cases in which the shrinkage was determined show that it was over 34 per cent in 86 days at 86° F., the higher storage temperature. The thickness of the skin of the fruit and the percentage of peel decrease markedly in ventilated warm storage. This, of course, makes impossible the calculation of the actual shrinkage of the pulp. The percentage of total sugar in the pulp is in all cases higher after storage. This increase is due in most cases to an increase in the reducing-sugar content, for the percentage of cane sugar remains about the same in all analyses. It is quite possible, in spite of the apparent increase in sugar content, that some of the sugar originally present in the fruit actually disappears during storage.

Another series of experiments was carried out in which fruit from the second picking of the two "common Florida" trees was placed in the warm room at 70° F. after it had remained in common storage 51 days. The fruit was stored in cans and boxes, as in the experiments just described. The results are given in Table VII.

From Table VI it is apparent that fruit removed from common storage and placed at a higher temperature behaves the same as fruit stored at the higher temperature throughout the season. The findings in this series are then mostly corroborative.

In ventilated packages there was, in some cases, an apparent increase in acids, and in others the acid content was a little less. If the exceedingly high percentage of shrinkage is taken into account, the results seem to indicate that there is no actual increase in the amount of acid during storage and that there may be a decrease as compared with the amount originally present. The sugar content of the fruit stored in unventilated packages shows always a decrease in the percentage of total sugars present, while in ventilated storage the increase in sugar content is in no case more than sufficient to account for the probable

shrinkage of the pulp. In one case, tree 1, stored 67 days, the sugar content is less after storage, probably because of variation in the samples. The results indicate that there may be a slight decrease in sugar at the higher storage temperatures.

TABLE VII.—*Percentage of sugar, acids, dry matter, shrinkage of fruit, peel, and thickness of the peel of "common Florida" grapefruit stored 51 days in common storage then placed in warm storage*

TREE 1, SECOND PICK

Time of sampling.	Acids as citric.	Sugars as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
After 42 days in common storage	1.11 1.22	3.24	2.45	5.69	.....	.....	24.5	5.33
After 51 days in common storage; 29 days at 70° F., ventilated	1.13	3.43	2.56	5.99	.....	10.8	20.5	3.57
After 51 days in common storage; 29 days at 70° F., unventilated	1.02 1.01	3.20	2.38	5.58	.....	1.2	23	5.76
After 51 days in common storage; 67 days at 70° F., ventilated	1.15 1.11	3.62	1.94	5.56	9.44 9.34	20.2	17.5	2.70
After 51 days in common storage; 67 days at 70° F., unventilated	.99 1.03	3.21	2.17	5.39	9.45 9.64	3.3	26.2	.....

TREE 2, SECOND PICK

After 42 days in common storage	1.06 1.07	3.55	2.22	5.76	9.43	.....	24.3	.....
After 51 days in common storage; 28 days at 70° F., ventilated	1.12 1.10	3.76	2.28	6.04	9.61 9.40	11	19.9	3.35
After 51 days in common storage; 28 days at 70° F., unventilated	1.02 1.03	3.32	1.99	5.31	10.1 10.42	1.1	22.8	4.88
After 51 days in common storage; 67 days at 70° F., ventilated	1.12	3.65	2.17	5.82	10.34 10.01	23	7.5	2.50
After 51 days in common storage; 67 days at 70° F., unventilated	.99 .96	3.26	2.20	5.46	9.65 9.32	4.8	24.4	5.30

There is a marked difference in the shrinkage of the fruit and percentage of peel as well as in thickness of the peel in the ventilated and unventilated packages, the shrinkage being around 4 per cent in the unventilated fruit for 67 days and from 20 to 23 per cent for comparable lots stored in ventilated packages. The peel, as would be expected, becomes very much thinner in the fruit stored in ventilated storage.

There is a marked increase in the percentage of dry matter in the pulp of the fruit stored in ventilated storage, while that of fruit in unventilated packages remains practically constant.

To determine the effect of cold storage followed by warm storage upon the keeping quality of the fruit and also to obtain more data as to the acid-sugar changes, "common Florida" grapefruits of the first pick, which had been maintained at 32° F. for 61 days were removed, weighed, and placed in boxes at 70°. The analyses of this fruit after 46 days at 70°, as compared with the analyses of comparable lots from 32° at the time the fruit was placed in the warm chambers, are given in Table VIII.

TABLE VIII.—*Percentage of sugars, acids, dry matter, shrinkage of fruit, peel, and thickness of peel of "common Florida" grapefruit stored in cold storage 61 days and removed to warm storage for a period*

TREE 1, FIRST PICK

Time of sampling.	Acids as citric.	Sugar in pulp as dextrose.			Dry matter.	Shrinkage of fruit.	Peel.	Thickness of peel.
		Reducing.	Sucrose.	Total.				
								Mm.
After 60 days at 32° F.	0.92 .93	2.71	2.35	5.06	8.06	.....	.....	6.13
After 61 days at 32°; 46 days at 70°, ventilated.	.99 .97	2.91	2.39	5.31	9.86 9.80	24.1	27.3	2.70

TREE 2, FIRST PICK

After 61 days at 32°.	0.92 .92	2.63	2.43	5.06	8.60 8.22	.....	.....	5.98
After 61 days at 32°; 46 days at 70°, ventilated.	1.01 1.05	3.18	2.19	5.37	9.24 9.32	17.4	21.1	3.10

It is evident from Table VIII that there is an apparent increase in acidity, as was the case in most of the other warm-storage experiments. The total sugar content is somewhat increased, though less proportionally than the acid content. The percentage of dry matter is increased markedly, the shrinkage at the high temperature is very marked, the percentage of peel decreases, and the peel becomes thinner, the fruit behaving much as in all the warm-storage experiments.

It seems probable that there was in these experiments a decrease in the sugar during the period of warm storage, while the amount of acids remained about the same. The fruit compared very favorably in analyses with the grapefruit from the warm-storage experiments, the results of which are given in Tables V to VII.

GENERAL DISCUSSION

While this investigation is primarily concerned with the acid and sugar changes in the fruit, some data were obtained as to the general appearance and attractiveness of fruit stored at the various cold-storage temperatures and also at common storage.

The fruit will apparently keep for a longer period in cold storage than in either common or warm storage. In the first place, the losses from decay caused by microorganisms are much less in the cold-storage temperatures. In the second place, the shrinkage in cold storage is much less than in warm, ventilated storage or in common storage. A high percentage of the fruit rotted in warm, unventilated packages. A high degree of humidity is necessarily maintained in this storage, which is very favorable to the growth of various fungi which break down the fruit. There is, therefore, much loss. The fruit which does survive this treatment is, however, very attractive in appearance and has an excellent flavor. In the third place, the life of the fruit is apparently lengthened in cold storage—that is, the average fruit apparently tends to break down more quickly when maintained at temperatures above 40° F. than when stored at lower temperatures.

An undesirable feature of cold storage is the breaking down or pitting of the peel at the temperature of 40° F. or lower. This breaking down of the peel begins as a slightly sunken spot, which increases in size and becomes brown in color. The sunken portions are usually not more than ½ inch in diameter, but several may coalesce, making a large sunken area of dark-brown tissue. This does not extend into the pulp, and the flavor is apparently unaffected, but the fruits are rendered unsightly. In these experiments no pitting was noticeable on the fruit stored at the two warm-storage temperatures or in common storage. It occurred only on the fruit stored in the three cold storages. In these temperatures the fruit at 40° was most seriously affected. There was somewhat less pitting on the fruit in the 36° storage and only a little on fruit at 32°.

The flavor of the fruit improves in cold storage. The fruit is sweeter, as is obvious from the fact that the sugar content of the pulp is higher and the acid content lower. The fruit is apparently not so bitter after storage, which may be due to the breaking down of the naringin in the pulp. Zoller (*11*) has shown that this glucoside breaks down in the peel during storage. The fruit improves in taste more rapidly at high storage temperature than in cold storage, which is to be expected, inasmuch as the changes are more rapid in warm storage. After longer storage, however, the fruit in cold storage attains the excellence brought about more quickly at a higher temperature.

The experiments in which the fruit was removed from storage at 32° F. after 60 days and stored at 70° for 46 days (Table VIII) indicate that the grapefruit does not deteriorate rapidly after removal from cold storage. The fruit compared very favorably with fruit that had been stored at 70° from the beginning of the storage period.

From the data shown in Tables I to IX, there is no question but that the titratable acids in the fruit decrease after the fruit is removed from the tree and placed in cold storage, which is in accord with the behavior

of the acids in apples, as found by Bigelow, Gore, and Howard (*2*), and others, Bigelow and Gore on peaches, (*1*) and in pears by Magness (*6*).

The sugar content apparently does not decrease appreciably in cold storage, though definite evidence on this point is lacking. The shrinkage of the peel and pulp may not be proportional, so that an accurate determination of the original weight of the pulp is impossible. There is indication that the sugar content decreases slightly in warm storage if the shrinkage of the fruit is taken into consideration. There was in no case evidence of a markedly increased sugar content in the fruit, mentioned by Zoller (*11*). There is considerable variation in individual fruits, and it is possible that this would account for the increase in sugar content which he found. In the preliminary experiments, the results of which are given in Tables I and II, it is shown that there is a marked increase in the acid-solids ratio after storage at 86° F. This increase in amount of soluble solids is undoubtedly due mainly to the loss of water from the pulp and a concentration of the juice. While acid-solids determinations were not carried out in the later experiments, the results of the sugar and acid determinations show that a similar condition would hold for fruit stored at the cold-storage temperatures, though possibly not for fruit stored for long periods at the higher temperatures used.

In conclusion, it has been shown in this investigation that the acid content of grapefruits decreases in cold storage. There is an apparent increase in sugar content in cold storage, calculated to percentage of pulp, which seems to be due to loss of water from the fruit. The dry matter increases during storage. The shrinkage of the fruit runs from 5 to 8 per cent in cold storage to around 23 per cent in warm, ventilated storage.

Fruit was kept in cold storage for about six months. The best storage temperature seemed to be 32° F., for at this temperature the pitting was much less marked. Pitting of grapefruit does not apparently develop at high temperatures but occurs only on the cold-storage fruit. Grapefruits do not keep so long in common storage or warm storage as in cold storage. There is much more loss from decay at the higher temperatures.

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A BACTERIOLOGICAL STUDY OF CANNED RIPE OLIVES

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As a result of the first of the recent series of outbreaks of botulism traceable to the consumption of ripe olives infected with *Bacillus botulinus*,[2] many lots of canned ripe olives were collected by inspectors of the Bureau of Chemistry for bacteriological examination. These were obtained, for the most part, from various retail and wholesale houses in all parts of the country and bore the label of the same company as did those responsible for the fatalities. While the primary object of the investigation was the detection of the presence of *Bacillus botulinus*, this object was extended to include a study of the types of microorganisms responsible for the spoilage and also to determine whether viable microorganisms might be encountered in apparently normal containers. The containers subjected to examination included all sizes of both cans and glass jars. Some were apparently normal while others were swelled or obviously spoiled.

In the bacteriological examination of these samples the following procedures were adopted as a routine. All containers were opened with usual aseptic precautions, and 1.5 to 2 cc. of the liquor were withdrawn by means of a sterile pipette. Approximately 0.5 cc. of this was spread over a dextrose agar slant (for aerobes), and the remainder was then run into a tube of infusion broth under oil. This medium was a 0.2 per cent dextrose beef infusion broth (P_H 7.4 to 7.6). It was covered before autoclaving with a layer of liquid petrolatum. In place of this medium there was occasionally used a 2.0 per cent dextrose-beef infusion broth, similarly covered with a layer of oil and containing a small piece of meat. In most cases a piece of olive was removed with sterile knife or forceps and was transferred to the dextrose broth tube. Incubation was at 37° C. In addition, notes were kept on the condition of the container, whether normal, swelled, etc., and also on the odor. Cans which were obviously leaking were discarded.

It is realized that for the sake of completeness it would have been desirable to have included a greater variety of culture media and several

[1] The author wishes to express his appreciation of the valuable criticism and suggestions given by Dr. Charles Thom, of the Microbiological Laboratory.

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temperatures of incubation. The number of samples, as well as the urgency of the examination, however, forbade any elaborate series of tests. The total number of cans and glass jars, both normal and spoiled, which were cultured by various members of this laboratory, together with the number showing the presence of living organisms, is summarized as follows:

Experiment with cans

Exp. No.	
I.	Number of normal cans cultured 181
	Of this number 173 were sterile, while 8, or 4.4 per cent, were found to contain viable microorganisms.
II.	Number of "swelled" or "springy" cans cultured 157
	Of these 154 contained living microorganisms, while 3 were apparently sterile (2 of these 3 were "springers," the other was a "hard swell").
	Total number of cans cultured 338

Experiment with glass containers

Exp. No.	
I.	Number of containers normal in appearance and odor 116
	Of this number 105 were sterile, while 11, or 9.5 per cent, revealed the presence of living microorganisms.
II.	Number of containers obviously spoiled or of bad odor 26
	All of these 26 gave positive cultural results.
	Total number of glass containers cultured 142
	Total number of cans and glass containers cultured 480

Thus, it is seen that all the obviously spoiled glass jars, and, with one exception, all the swelled cans revealed the presence of living microorganisms. On the other hand, the normal containers were, for the most part, sterile. In this connection it is interesting to note that 4.4 per cent of the normal cans were found to contain viable organisms, while in the normal glass containers the proportion was decidedly higher, namely, 9.5 per cent. Of the 157 swelled or "springy" cans, all but three gave positive cultural tests. Two of these three were "springers," due probably to imperfect exhausting, and were no doubt otherwise normal. The third was a "hard swell." Whether the failure to obtain living organisms from this one can was due to lack of a greater diversity of culture media or whether the causative organisms had been killed as a result of their own metabolic products is not known.

Of the total of 480 containers examined bacteriologically, 117 of those which had yielded positive cultural tests were studied further to gain some knowledge of the types of organisms present. As a rule, extensive cultural and biochemical tests were omitted, and merely the general type or group to which the organisms belonged was determined. A summary of the types obtained from the 117 containers thus studied is shown below. The figures indicate the number of times each was encountered.

Types of organisms found

Colon group	81
Colon group, sluggish liquefaction of gelatin (*Bacillus cloacae*)	4
Bacterium fluorescens (liquefying)	2
Proteus	3
Other Gram-negative, non-spore-forming bacilli, not identified	5
Gram-positive, aerobic, spore-forming bacilli, gelatin liquefiers—	
Bacillus cereus type	3
Bacillus mycoides type	4
Bacillus mesentericus type	6
Type not determined	19
Slender, Gram-positive, aerobic or faeultative anacrobic bacilli, oval terminal spores, gelatin not liquefied	10
Gram-positive diplococci	31
Gram-positive staphylococci	10
Spore-forming, obligate anaerobes	6
Yeasts	3
Mold (*Aspergillus terreus*) [1]	1

In addition to these, *Bacillus botulinus* was found in 7 of the spoiled glass jars. A report of the findings of this laboratory with respect to *Bacillus botulinus*, both from the material obtained in the open market and from specimens received from the poisoning cases, has been made the subject of another paper.[2]

The large proportion of non-spore-forming organisms, particularly of the colon group and the Coccaceae, was indeed surprising. Many of the cultures of the colon group when first isolated exhibited a delayed fermentation of lactose somewhat similar to that reported by Bronfenbrenner and Davis,[3] though not so marked. In lactose broth, acid formation was delayed from 48 to 72 hours, while gas was produced only after 3 to 5 days' incubation. After several successive transplants in lactose broth, fermentation of this sugar was markedly accelerated.

Although some of the organisms obtained were placed without difficulty in their proper groups, others were not identified by the limited number of cultural tests employed, and these are designated in the foregoing list by their chief cultural characteristics or morphology. Many Gram-positive diplococci were encountered. These exhibited a distinct lance-shaped appearance in liquid media, with occasional short chains of three or four elements. On dextrose agar slants the individual colonies appeared as minute white pin points. In beef infusion broth under oil

[1] For identification of this species the writer is indebted to Dr. Margaret B. Church, of the Microbiological Laboratory.

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the growth was fairly luxuriant, producing a distinct cloudiness after 24 hours' incubation. The other type of Gram-positive coccus encountered grew more luxuriantly on plain agar slants and was found upon staining to occur in irregular clusters. The several obligate anaerobes were inoculated into milk and into the meat medium of Holman.[1] One culture digested the meat with a distinct putrefactive odor. The remaining five caused neither putrefaction of meat nor stormy fermentation of milk. Dextrose was attacked with acid and gas production. Up to the present time they have not been studied further.

FLORA OF SWELLED CANS.—The flora of swelled cans was found to consist largely of members of the colon group, for of 85 swelled cans studied this group was obtained from 75, and from 40 of these in apparently pure culture. In the others they were found in mixed culture with the several types of Coccaceae, the aerobic, Gram-positive, spore-forming bacilli, or, more rarely, with an obligate anaerobe, with Proteus, or with a yeast. In three instances spoilage, with resultant swelling of the can, was evidently due to spore-forming anaerobes only. In one instance Proteus was found in pure culture. A few of the swelled cans yielded cultural results from which no evidence could be gathered as to the type of organism causing gas formation within the can. Thus, an aerobic, spore-forming, Gram-positive rod was the only type obtained from 2 swelled cans, while from 2 others Gram-positive cocci were obtained in pure culture. Since none of these organisms attacked carbohydrates[2] with gas production, it is evident that the gas-producer had disappeared or was overlooked.

NORMAL CONTAINERS.—As previously shown, 8 normal cans and 11 normal glass containers were found to contain living microorganisms. Four of these 8 normal cans yielded cultures of the colon group. The others contained cocci and several types of aerobic, spore-forming bacilli. The finding of members of the colon group in 4 of the normal cans was rather surprising. Evidently for some unknown reason the bacilli failed to multiply to any extent in these cans. Without exception, the types encountered in the normal glass jars were aerobic, spore-forming, Gram-positive rods. Several were identified as *Bacillus mesentericus* and one as *Bacillus cereus*.

SPOILED GLASS JARS.—The flora of the spoiled glass jars was as a rule more varied and complex than that of the swelled cans. The contents of several jars were obviously spoiled and disintegrated to a mushy consistency with a disagreeable odor, unrecognizable as that of olives. These yielded a diversity of types of which the following are illustrative:

[1] HOLMAN, W. L. THE VALUE OF A COOKED MEAT MEDIUM FOR ROUTINE AND SPECIAL BACTERIOLOGY. *In* Jour. Bact., v. 4, no. 2, p. 149–155. 1919. References, p. 155.

[2] Chemical analyses by the Food Control Laboratory of the Bureau of Chemistry showed the liquor in which the olives were packed to contain from 0.16 to 0.23 per cent reducing sugars after inversion, expressed as percentage of invert sugar.

1. Putrefactive anaerobe which digested a cooked meat medium with a putrefactive odor, an aerobic Gram-positive, spore-forming rod, and an unidentified Gram-negative bacillus.

2. *Bacterium fluorescens liquefaciens*, Proteus, aerobic Gram-positive, spore-forming bacillus, and an unidentified non-gas-producing Gram-negative bacillus.

3. Staphylococcus, a yeast, and Gram-positive, sporing bacillus.

4. Gram-positive diplococci, colon group, *Aspergillus terreus*, and a Gram-positive, spore-forming rod.

No definite correlation between the odor of the spoiled samples and the type of organism contained therein was noted. The swelled cans from which the colon group only was obtained were recorded as possessing either a flat or slightly "off" odor—that is, they lacked the characteristic fragrant aroma of the first-class product. Since many of the sterile normal cans, particularly of certain brands, had a similar odor, it is doubted whether this condition can be ascribed solely to the metabolic activities of the colon group. Three cans containing spore-forming anaerobes possessed a disagreeable or rancid odor. The liquor, together with portions of the olives from several of the most offensive cans, was fed to guinea pigs without ill effects.

The large numbers and diversity of types encountered, particularly of the non-spore-formers, point to insufficient heating of the product. While it is realized that there may be a slight leakage along the seam of the can immediately after heating, and with subsequent closure, it would seem improbable that this could account entirely for the results obtained in this investigation.

SUMMARY

(1) In the bacteriological examination of 480 commercial containers of ripe olives, living microorganisms were obtained in practically every instance from samples which were abnormal, as indicated either by a swelled condition of the container or a bad odor.

(2) Viable microorganisms were found in a small percentage of normal containers. These were either aerobic, spore-forming bacilli, cocci, or apparently dormant members of the colon group.

(3) A study of the organisms encountered in the spoiled samples showed a great diversity of types, among which the colon group predominated.

RELATION OF THE SOIL SOLUTION TO THE SOIL EXTRACT

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Modern views of soil fertility recognize the general principle that plants derive their immediate supply of inorganic elements entirely from the soil solution. It has also been proved that the soil solution is subject to highly significant fluctuations. The concentration and composition of the soil solution may undergo very great alterations as a result of seasonal changes, crop growth, activities of microorganisms, rainfall, fertilization, etc. The evidence supporting this point of view is now too strong to admit of any doubt. It is justifiable to assume, therefore, that further progress in the study of the soil as a medium for plant growth will depend upon an increased knowledge of the soil solution, particularly in its dynamic relations to the soil mass, to the plant and microorganisms, and to the application of fertilizing materials.

Experimental work on the soil solution immediately encounters a formidable obstacle in the difficulty of separating from the soil the solution which provides nutriment to the plants. When the soil contains moisture in percentages most suitable for plant growth, the solution is held by the soil particles with such force that no ordinary means will serve to effect a separation. This fact is well recognized, and various attempts have been made to overcome the difficulties involved and to obtain the soil solution in an unmodified state. The most important developments in this phase of the work have been described by Morgan (*8*) [1] and by C. B. Lipman (*7*). It is yet too early to state any final conclusions based on data obtained by these methods, but their further perfection may lead to the attainment of most essential information. A considerable advance in our ideas concerning the soil solution has already resulted from the application of the freezing-point method to soils, as first described by Bouyoucos and McCool (*4*). However, the study of the soil solution in its relation to plant growth is so fundamental that it should be approached from every possible angle, with the hope that eventually we may possess an adequate understanding of the nature of the nutrient medium in the soil and of the modifications produced in this medium by various treatments.

Since the soil solution is constantly undergoing modification, the investigator is often required to make numerous determinations at frequent

[1] Reference is made by number (italic) to "Literature cited," p. 394-395.

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intervals during the growing season at least. This introduces certain practical difficulties in the application of presssure methods. The freezing-point method is most rapid and convenient as a means of studying the approximate total concentrations, but it can not give any information concerning the individual solutes. The method of water extraction has been used rather frequently in past investigations with the intent to determine the amounts of plant foods available to the plant. One of the writers (9) has carried out an extensive investigation in which

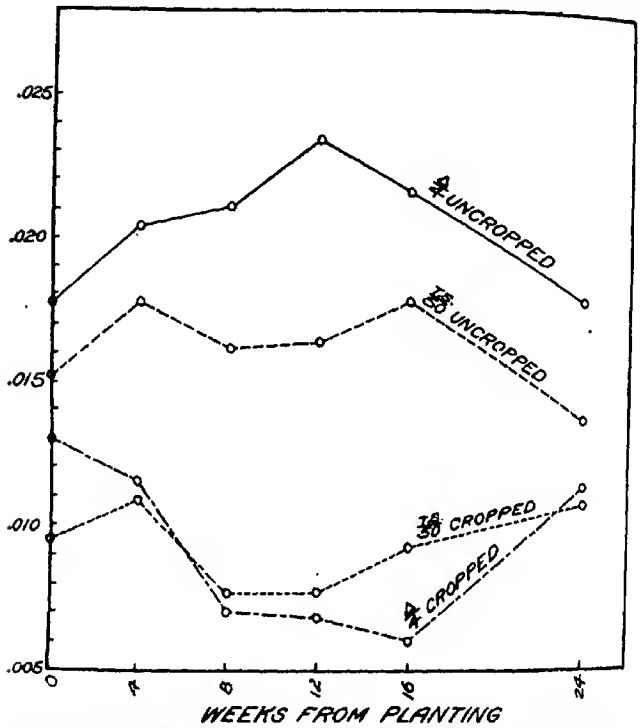

FIG. 1.—Graph showing relation of freezing-point depressions in soil (calculated to 22 per cent moisture) to total solids extracted by 5 parts of water to 1 of soil. Individual data from six soils composited.

the very significant effect of season and crop growth on water extracts of soils was made clear. At the same time the freezing-point method of Bouyoucos and McCool was applied to the soils under investigation, and a general agreement was noted between the values obtained by this method and by the water-extraction method (6). Thus the effect of the crop in diminishing the concentration of the soil solution was definitely shown by both methods. At the present time the study of water extracts offers such promise that it has seemed highly important to attempt

to throw further light on the relation between the soil extract and the soil solution. The value of the determination made by the water-extraction method rests primarily on the assumption that a logical relationship exists between water extracts and the soil solution.

In the articles referred to above considerable data were presented to show that in general the larger fluctuations in the total solids found in 1 to 5 water extracts occurred coincidently with similar fluctuations in the freezing-point depressions of the moist soil. Later much more ex-

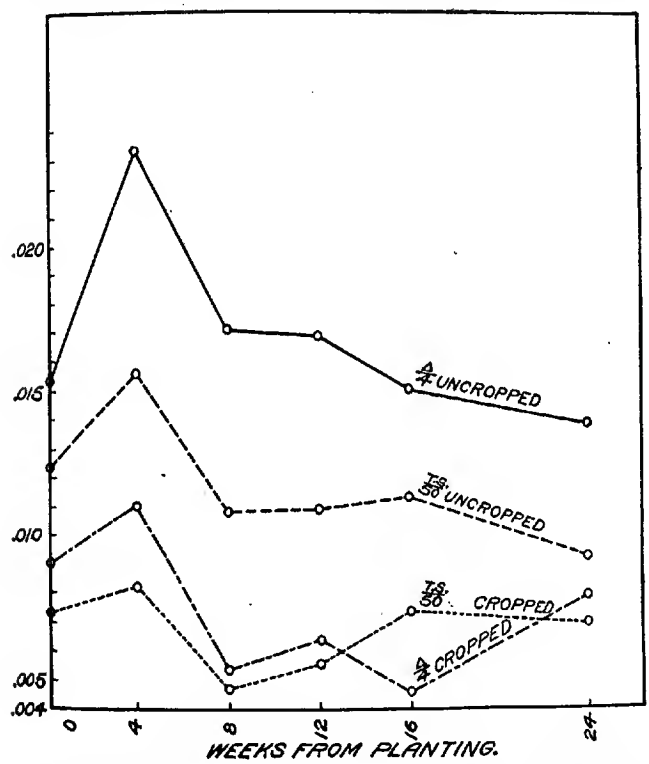

FIG. 2.—Graph showing relation of freezing-point depressions in soil (calculated to 17 per cent moisture) to total solids extracted by 5 parts of water to 1 of soil. Individual data from seven soils composited.

tensive observations were made on this important question, and the data have been plotted in two graphs (figs. 1, 2), one for the group of silty clay loam soils and the other for the various fine sandy loams, the soils being the same as those described in a previous article by Stewart (9). The data for the individual soils have been composited for the present paper. In these graphs the determinations of total solids and freezing-point depressions are plotted for various time intervals, for the soils in both the cropped and uncropped condition. The correlation between

the curves for total solids and freezing-point depressions is on the whole excellent, considering the technical difficulties involved. Chief among these is the uncertainty concerning the free and unfree water in the soil, which, as Buoyoucos (*1*) has clearly shown, markedly affects the concentration of the soil solution. While all the values have been calculated to the same moisture basis, it is not to be expected that this can be done with a high degree of accuracy, since the percentage of unfree water may vary with different moisture contents and perhaps with different concentrations of the soil solution. In both groups of soils there is a somewhat marked divergence between the curves for total solids and freezing-point depressions at a period beginning about 10 weeks after planting the crop. This can reasonably be explained on the basis of certain observations reported in former articles (*6*, *9*). It was shown in these that a larger quantity of very slightly soluble material was dissolved from a soil by a given proportion of water when the soil solution had reached a low concentration as a result of absorption of solutes by the plant. At a certain period, therefore, the cropped soil will yield a higher percentage of dissolved material (not part of the actual soil solution) as compared with earlier periods. This means that the extractions of the cropped and uncropped soils are not on exactly the same basis at all times, and it might be predicted that at the period of low concentration in the cropped soil the proportion of dissolved substances would increase. The inference is substantiated by the experimental data. This generally neglected phenomenon of the effect of the solutes already present in the soil solution in depressing the solubility of substances dissolved from the soil mass by water is thought to be of considerable importance in all studies on soil equilibria by means of water extracts. Finally, it should be emphasized that at no time is there any indication that conclusions based on the water extracts would lead to an erroneous estimate of the general relation between the soil solutions of cropped and uncropped soils. As the authors have pointed out before, the actual difference would tend to be of greater magnitudes than those calculated from the results on water extracts.

When a 1 to 5 extract of soil is made with distilled water, the quantity of total solids is from 1.5 to 5 times that present in the soil solution, as calculated by the freezing-point method. By the latter method we can calculate the total concentration in the soil solution; but this does not enable us to determine whether or not the relation between the elements in the soil solution is at all similar to that in the soil extracts. Another type of experiment is necessary to give evidence on this point. It was suggested that such evidence might possibly be obtained by determining the concentration and composition of a solution which would remain unchanged when in contact with the soil mass. In other words, if one passed through a sample of moist soil a solution having the

same concentration and composition as the soil solution already present, then it may be assumed that the resultant extract would have the same composition and concentration as the original solution. On the other hand, if the solution used were of different concentration or composition a readjustment of the equilibrium should take place so as to produce a different extract. It was decided to attempt an experiment based on this hypothesis.

Obviously, the preparation of a solution having the same composition and concentration as the soil solution is a matter of great difficulty. The only feasible scheme seemed to be the use of a soil extract concentrated to a point where it would have the same concentration as the soil solution, this concentration being determined by the method of Bouyoucos and McCool. It was reasonable to assume that in such a solution there would exist, between some of the most important elements, a relation very similar to that found in the actual soil solution—that is, the solution of the free water with the soil at approximately optimum moisture content. In order to limit as far as possible the quantity of solutes dissolved from the soil mass, an extract was made with cold water, and only ½ part of water was used to 1 part of soil. The time of contact was limited to that necessary for complete admixture. Filtration was made through a Buchner funnel, and final clarification was effected with the use of a Pasteur filter. A separate portion of the soil was then made up to its optimum water content, and the freezing-point depression was carefully determined. The extract of the soil made in the manner described was then concentrated on a hot plate, meanwhile passing through the solution a stream of carbon-dioxid gas in order to prevent any precipitation. Finally the volume of the concentrated extract was adjusted with distilled water so that it had exactly the same freezing-point depression as that of the moist soil. This solution was used in extracting the moist soil (1 part of soil to ½ part solution). Careful analyses were made of the extract before and after contact with the soil, and the results were compared.

Before the data are considered it should be recalled that ordinarily in a water extraction from 2 to 5 times as much total solids are dissolved as are actually present in the soil solution, and this is true with the extractions now considered. Under certain conditions, however, it is possible to obtain an extract which contains a comparatively small quantity of dissolved substances in addition to that originally present in the soil solution, as indicated by the method of Bouyoucos and McCool. For example, a sample of soil 9, having a freezing-point depression of 0.148° C. at 17 per cent moisture gave in a 1 to ½ extract only about 1.16 times the quantity of total dissolved solids equivalent to this depression. In this case the unfree water was determined directly by dilatometer measurements (*1*). Such a result apparently can be obtained only with

a soil having a low percentage of colloidal material and having a fairly high concentration in its soil solution, which exercises a repressive effect on the solubility of certain soil constituents as previously explained.

In Table I the results of the equilibrium studies with three different soils are presented. Comparisons are made between the composition of the concentrated extracts and the same extracts after treatment with the soil. It will be noted that the total concentration has suffered practically no change, as shown by the freezing-point depressions, conductivity determinations, and proportion of total solids. Also, the concentrations of potassium, magnesium, calcium, nitrate, and sulphate agree within the limits of experimental error. The agreement for sodium is less perfect, but considering the small quantities involved the differences are also probably within the limits of error. In one case more phosphate is found in the re-extract, and in two cases the agreement is fairly close. In one case the two silica determinations agree almost perfectly, and in two cases silica seems to have been retained by the soil. It is very difficult to explain the action of this radicle, first because of the chance of contamination of the solution from glass vessels and secondly because of the numerous types of silicates possible with varying proportions of silica.

While the agreement between the extracts and re-extracts is on the whole remarkably close, it might be objected that the conditions for the attainment of equilbrium were inadequate and that another extract having a different composition might also remain unchanged by the soil. In order to test this possibility extracts were made of soils 9 and 15 in the previously described manner, and then potassium sulphate was added to the extracts so as to double approximately the concentration of potassium. These modified extracts were then concentrated until they had the same osmotic value as the soil solutions, and re-extracts were made as in the first experiment. The composition of the different solutions is given in Table II. It is evident that in this experiment the soil has had a marked effect on the extract. There is very much less potassium in the re-extract than in the original extract, but the decrease of potassium is accompanied by an increase in the quantity of calcium and in one case of sodium. In one case there is a slight decrease of sulphate. The other elements are not greatly changed, nor is the total concentration very different in the two cases. It seems clear that a rearrangement of the solutes has taken place in this case which did not occur in the first experiment. In other words, the extract introduced was different in composition from the soil solution already present, with the result that certain chemical reactions took place forming an entirely new soil mass—soil solution system.

TABLE I.—*Extraction of soil with its concentrated extract*

Soil No.	Description of extracts.	Freezing-point depression.	Specific resistance.	Composition of extracts.									
				Total solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Sodium (Na).	Nitrate (NO_3).	Phosphate (PO_4).	Sulphate (SO_4).	Silica (SiO_2).
		°C.	*Ohms.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1C	Concentrated extract	0·064	520	1,944	50	148	74	30	348	3	183	61
	Same after passing through soil	·063	550	1,904	45	148	74	31	348	4	183	33
9	Concentrated extract	·041	610	1,506	46	228	27	41	366	3	116	53
	Same after passing through soil	·043	590	1,688	41	264	27	43	384	4	108	25
15	Concentrated extract	·006	250	2,640	84	308	61	48	558	6	168	26
	Same after passing through soil	·064	260	2,086	72	320	58	34	552	6	170	25

TABLE II.—*Extraction of soil with concentrated extract containing added potassium sulphate* (K_2SO_4)

Soil No.	Description of extracts.	Composition of extracts.								
		Total solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Sodium (Na).	Nitrate (NO_3).	Phosphate (PO_4).	Sulphate (SO_4).	Silica (SiO_2).
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
9	Concentrated extract plus potassium sulphate	1,652	76	212	33	122	312	3	166	40
	Same after passing through soil	1,896	52	280	31	129	333	4	161	40
	Increase or decrease in concentration	+244	− 24	+ 68	− 2	+ 7	+21	+1	− 5	.....
15	Concentrated extract plus potassium sulphate	2,408	277	236	41	72	.....	4	413	24
	Same after passing through soil	2,592	129	300	41	109	.....	3	369	22
	Increase or decrease in concentration	+184	−148	+ 64	.....	+ 37	.....	−1	− 44	− 2

All the experiments just discussed would seem to justify the conclusion that in a concentrated extract the relation between the various elements may be very similar to that existing in the soil solution. In seeking an explanation of the results it is first essential to describe the components which probably enter into a water extract of a soil. In the first place, these would include the constituents of the soil solution diluted by the added water. This diluted soil solution would then tend to bring into solution constituents which were not present in the soil solution itself. Thus, in no case would the solvent be pure water but rather a solution the composition and concentration of which would vary with the soil solution. It is plausible to assume that the solvent thus formed would bring into solution principally either "adsorbed" salts or easily soluble chemical compounds, originally derived from the more resistant minerals. Finally, a certain quota of this very slightly soluble material would come into solution, and the total quantity dissolved would depend at least in part on the total volume of water as well as on time and temperature. This latter fraction of the soil extract would ordinarily form only a small portion of the total dissolved material. Evidence for this view has been presented previously (*6*, *9*) and is also upheld by certain experiments of Bouyoucos with regard to the solubility of soil minerals (*2*). It would follow, therefore, that if the adsorbed or immediately soluble material has the same relative composition as that already present in the soil solution, then the water extract might also retain similar relations. It is impossible at present to obtain direct evidence to this effect, but an experiment was carried out from which certain inferences may be drawn. A large quantity of moist soil (silty clay loam 1) was placed in a Buchner funnel and leached with the least possible proportion of distilled water, about ⅛ part of water to 1 part of soil. Two subsequent leachings were made with similar proportions of water. These three extracts were then analyzed for the most important elements, and the ratios

between them were calculated by dividing the concentration of each element by the sum of the concentrations of all the elements determined (Table III). The ratios were found to be very similar in the three extracts, the agreement for several elements being especially close in the first two extracts. In the first extract the larger proportion of solutes present were probably derived from the soil solution, while the subsequent extracts represented to a greater degree previously undissolved fractions of the soil. The results would, therefore, seem to indicate that in concentrated extracts there is a great similarity in composition between the soil solution and the extract containing the substances which immediately go into solution on the addition of a slight excess of water. Even with nitrate, which might be supposed to have such a high degree of solubility that the total quantity present would be contained in the soil solution, it is probable that a certain proportion is held in some adsorbed or undissolved form. If an extract of the soil be made, a readjustment takes place because of the great dilution of the soil solution, and the total quantity of adsorbed nitrate would be greatly diminished, even though the partition ratio between solution and soil remained constant. Thus, it is possible to extract nearly all the nitrate present but difficult or impossible to remove the last traces.

TABLE III.—*Composition of successive leachings of soil*

[8 parts soil to 1 part water.]

Solute.	First leaching.		Second leaching.		Third leaching.	
	Concentration of solution.	Ratio of individual solutes to total.	Concentration of solution.	Ratio of individual solutes to total.	Concentration of solution.	Ratio of individual solutes to total.
	P. p. m.	*Per cent.*	*P. p. m.*	*Per cent.*	*P. p. m.*	*Per cent.*
Nitrate (NO_3)	425	57.0	195	53.0	133	46.0
Calcium (Ca)	90	12.1	45	12.3	46	15.8
Magnesium (Mg)	88	11.8	43	11.8	34	11.6
Potassium (K)	22	3.0	15	4.1	13	4.4
Sulphate (SO_4)	121	16.2	67	18.4	66	22.6

With regard to phosphate the case is not so clear. Most of the extraction studies described in previous articles have indicated that the various extracts are saturated with respect to phosphate. Thus, if the extract were concentrated without precipitation the concentration of phosphate should be considerably greater than in the soil solution. Since, however, the adsorption or precipitation of phosphate by the soil is a relatively slow process, in the present experiment the time may have been insufficient for readjustment of the equilibrium. From our previous experiments we should be inclined to infer that the concentration of phosphate in the soil solution is usually very low, but that immediate replacement occurs as phosphate is absorbed by the plant, thus producing a constant concentration of phosphate over long periods of time.

That there exists some sort of definite and reversible state of equilibrium between the soil mass and the soil solution for any given set of conditions is suggested by another experiment. Two soils were treated with water in the proportion of 1 part of dried soil to 1 part of water. After the soil and water were thoroughly mixed the resultant mixtures were allowed to dry at room temperature until they reached the optimum moisture content. Freezing-point depressions were then made and compared with determinations made on samples of the same soils simply moistened to optimum water content. The data given below show that the agreement is, at least in these two cases, almost perfect.

TABLE IV.—*Freezing-point depressions of soil at optimum moisture content and of treated soil evaporated to optimum moisture content*

Description of soil.	Freezing-point depressions.
	°C.
Soil 1C at optimum moisture content	0.063
Soil 1C after mixing 1 to 1 with water and allowing to evaporate to optimum moisture content	.062
Soil 9 at optimum moisture content	.045
Soil 9 after mixing 1 to 1 with water and allowing to evaporate to optimum moisture content	.047

In other words, although several times as much material was brought into solution as was contained in the soil solution at optimum water content when the excess water was added, these dissolved substances were immediately removed from solution on lowering the moisture content. This, of course, does not mean that the concentration of the soil solution may not easily be altered by the addition of soluble salts, as will be discussed presently.

If the general method of studying soils by means of their water extracts is of value, then it becomes of considerable importance to determine the most suitable conditions for making the extract. The technic might be based on either one or two general objectives, first the attainment of equilibrium (as nearly as possible final) for a given proportion of water, and, second, the limitation of the extract as far as was practicable to the material actually existing in the soil solution. In the first case a long period of contact and continuous shaking would be essential; in the second case the time would be limited to that necessary for complete admixture of soil solution and added water. In order to determine the magnitudes of dissolved substances under varying conditions, extracts of 3 soils were made by various methods as follows: (*a*) 1 part soil to 5 parts water, as described by the Bureau of Soils of the United States Department of Agriculture; (*b*) 1 part soil to 5 parts water, shaking for 1 week; (*c*) 1 part soil to 1 part water, as in (*a*); (*d*) 1 part of soil to 1 part water, shaking for 1 week.

In Table IV the results on extracts obtained by these different methods are presented, all calculated to parts per million of the dry soil, so that comparisons may be made on the same basis.

If the total solids are considered, it will be noted that the magnitudes are very similar except in the case of the 1 to 5 extract shaken for 1 week. More potassium is extracted by a 1 to 5 extract than by a 1 to 1 extract, but the quantities are essentially the same whether the time is 40 minutes or 1 week. The calcium, magnesium, and sulphate may be appreciably increased during the week's contact when the proportion is 1 to 5 but not when the proportion is 1 to 1. Nitrate is not greatly changed in a 1 to 5 extract by the increased time of extraction. In the 1 to 1 extract in one case of a heavy-textured soil there is a decrease after 1 week, and in another case of a light-textured soil there is an increase. Very probably biological action is concerned in these changes. Phosphate is increased markedly in the 1 to 5 extract as compared with the 1 to 1 extract.

Several fairly definite deductions may be drawn from the data just presented. When a smaller proportion of water to soil is used, as 1 to 1, there is only slight increase in dissolved substances with the period of 1 week as compared with a shorter period, although some changes in nitrate may result from biological action. There would not seem, therefore, to be any advantage in the longer period of contact; in fact the biological changes would make such a procedure undesirable. In the 1 to 5 extracts there is a significantly increased solution of various elements (particularly calcium and magnesium) during the period of a week. This must be due to the solution of soil minerals, more of which are dissolved in the 1 to 5 extract because of the greater dilution of the solvent, as previously explained. Phosphate is in a somewhat different category from the other elements in that the total quantity dissolved is somewhat directly dependent upon the volume of the solvent. As was stated before, to a certain extent the solution is always saturated with respect to phosphate.

TABLE V.—*Comparison of extracts of soil prepared by various methods*

Soil No.	Time of extraction.	Ratio of soil to water.	Composition of extracts calculated to basis of water-free soil.						
			Total Solids.	Potassium (K).	Calcium (Ca).	Magnesium (Mg).	Nitrate (NO_3).	Phosphate (PO_4).	Sulphate (SO_4).
			P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
10	40 minutes	1 : 1	680	24	62	26	80	1	60
	1 week	1 : 1	612	26	47	24	54	1	79
	40 minutes	1 : 5	636	38	47	25	128	6	.....
	1 week	1 : 5	1,034	37	74	33	126	5	81
9	40 minutes	1 : 1	510	16	68	7	68	2	27
	1 week	1 : 1	562	20	69	11	114	2	37
	40 minutes	1 : 5	503	33	70	10	114	7	29
	1 week	1 : 5	806	38	100	19	128	8	30
15	40 minutes	1 : 1	532	23	59	13	78	2	41
	1 week	1 : 1	524	30	51	16	63	1	45
	40 minutes	1 : 5	582	31	52	15	116	7	.....
	1 week	1 : 5	836	38	91	24	145	10	55

The application of the foregoing conclusions would seem to indicate that soil extracts should be made with a small proportion of water and for a short period. It would probably be desirable to use not more than 1 part of water to 1 part of soil, but in many cases this may be impracticable, so that 1 to 5 extracts must suffice. It is true that special studies of soil equilibria must take into account long-continued solvent action, but in attempts to gain some idea of periodic changes in the soil solution the technic should be directed toward lessening the solution of material not actually present in the soil solution. This aim is less possible of attainment in proportion as the volume of water or time of contact with the soil is increased. It is not evident that attempts to reach approximate final equilibrium by large excess of water or long shaking are likely to result in more accurate knowledge of the soil solution as it exists at any given moment. On the contrary, the increase in solutes is derived from substances not actually present in the soil solution, and their solubility is in part conditioned on the concentration of the soil solution, the variable under investigation.

In concluding this discussion it may be well to summarize briefly our present point of view with regard to the soil solution based on recent researches in this and other laboratories. All the evidence supports the general views expressed by Cameron (5) a number of years ago to the effect that soil phenomena must be considered as dynamic. His criticisms of the older methods of study by means of hydrochloric-acid extracts of soils, analyses of total quantities present in the soil, etc., are found to be entirely justified. It is now generally recognized, however, that Cameron's conclusions with regard to the nature of the soil solution were not sufficiently far-reaching. It is certain that the soil solution is not simply a solution saturated with respect to all the original mineral components of the soil and tending to approach a constant composition. The original soil minerals themselves doubtless have a very slight solubility in pure water, but the soil solution of a normally occurring soil is something quite different. The solvent is never pure water, but rather a solution of salts and organic matter, accompanied by carbon dioxid, oxygen, and other gases. The soil solution at any given moment is the resultant of the cumulative effect of the continuously varying solvent on the soil minerals. The actual concentration of the solution is governed principally by the equilibria existing between the dissolved substances and the immediately soluble or absorbed substances. It is possible that these latter may be removed almost completely from the soil mass by an excess of water. The soil solution in contact with the residual soil has a very low concentration, and this is not readily increased by the solvent action of pure water. To a lesser degree a similar state of affairs results when the dissolved or immediately soluble components of the soil are removed by a crop. This effect may be of long duration, or, on the other hand, the concentration of the soil extract with respect to many solutes

may easily be increased by the addition of soluble salts. Bouyoucos and Laudeman (*3*) have shown, moreover, that this increase of concentration occurs immediately and in most cases is not altered over a long period of time.

Theoretically, also, it is very apparent that the soil solution or extract may be increased in its concentration of a given element by the addition of a soluble salt. A simple case will illustrate this fact. A saturated solution of slightly soluble silicates of potassium, for example, can be prepared by shaking the finely divided minerals with water. The concentration of potassium in the solution is limited by the solubility of the components of this particular system. However, the addition of another component of different solubility, such as potassium chlorid, will increase the concentration of potassium in the solution, although the solubility of the potassium silicate may possibly be diminished because of the increased concentration of the potassium ion. In the same way the soil solution is saturated only with respect to the particular system existing at any given moment. In general it is not saturated with respect to any particular ion, so from theoretical considerations there is no reason to accept the earlier statements of Cameron that the chemical equilibria would require the precipitation of added salts with a tendency to maintain a constant composition in the soil solution. The fact that water extracts of soils become more dilute with each increase in the proportion of water used gives evidence to show that the solubility of the original soil minerals is not the chief factor governing the concentration of the soil solution.

Presumably in the actual soil solution the increase of concentration due to the addition of soluble salts will in part be limited by the removal from the dissolved to the absorbed phase. When an excess of water is employed, however, as in making an extract, nearly all of the added solutes will appear in solution or be represented by equivalent quantities of other substances, as is shown, for example, in the well-known exchange of bases. The total quantity of absorbed substances would be a function of the concentration of the surrounding solution, which would vary with the moisture content of the soil or volume of water used in making an extract. In extraction procedures there would occur, of course, a very great dilution of the soil solution. While the latter would be increased in concentration by the addition of soluble salts, the evidence at hand does not indicate that all the added salt would necessarily be effective in increasing the concentration of this soil solution even when the water extracts contained the total or equivalent quantities of the elements added. It is reasonable to assume, however, that the "adsorbed" substances are capable of easily replenishing the soil solution when its concentration is decreased as a result of withdrawals by the plant, new soil solution-adsorption systems being formed continuously during the season.

SUMMARY

(1) Seasonal studies on cropped and uncropped soils have shown that water extracts reflect the principal fluctuations taking place in the soil solution as indicated by the freezing-point method.

(2) A soil extract is composed chiefly of the solutes present in the soil solution plus substances dissolved from "adsorbed" or easily soluble components of the soil. This latter fraction of the soil extract is dependent in part on the concentration and composition of the soil solution, since the solutes of the latter exert a depressing effect on the solubility of certain soil constituents. This fact is believed to be of great importance in studies of chemical equilibria in soils.

(3) A new method is suggested for indicating the relations between the chemical elements in the soil solution. Extracts were prepared which did not change appreciably in composition or concentration on contact with the soil. The consideration of the equilibria involved suggests the probability that the ratios between most of the important elements are very similar in concentrated soil extracts and in the soil solution. It is concluded that analyses of suitable soil extracts and determinations of freezing-point depressions may frequently permit a calculation of the concentration and approximate composition of the soil solution.

(4) Various methods of making water extracts have been compared. The data obtained suggest that in seasonal studies extracts should be made with the smallest proportion of water to soil practicable and with the time of contact limited to that necessary for thorough admixture. In routine work 1 to 1 or 1 to 5 extracts are convenient and satisfactory.

(5) Further experimentation has confirmed previous conclusions that the soil solution fluctuates in composition and concentration with every environmental change and with crop growth.

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EFFECT OF SEASON AND CROP GROWTH ON THE PHYSICAL STATE OF THE SOIL

By D. R. HOAGLAND and J. C. MARTIN, *Division of Agricultural Chemistry, California Agricultural Experiment Station*

Investigations previously reported by this laboratory[1] have shown definitely that the soil solution is extremely variable in its composition and concentration, as indicated by water extracts or by the freezing-point method of Bouyoucos and McCool.[2] Recently McCool and Millar[3] in an extensive series of field studies have upheld this conclusion. In all these investigations it has been demonstrated that the absorption of solutes by the plant may have a very pronounced influence on the soil solution at certain periods and may bring about a very striking decrease in the concentration of nitrates and other constituents. Moreover, this condition may persist for a long time. During the course of our experiments it was noted that the state of dispersion of the colloidal matter in the various soils fluctuated in a most decided manner under the influence of the different treatments. It was decided, therefore, to make a systematic series of observations relating to this point.

The soils used were kept under controlled conditions in tanks as described by Stewart.[4] Both cropped and uncropped soils were compared under otherwise identical conditions. The principal measurements were made on a number of tanks of silty clay loam soil, clay in which various crops were grown—namely, corn, barley, potatoes, beans, and beets. There were three tanks of barley, containing, respectively, 24, 50, and 71 plants. All soils were kept at approximately optimum moisture content by the addition of distilled water. At frequent intervals during the growth of the crops samples of soil were taken for examination.

In order to study conveniently the changes in the water-soluble constituents, conductivity measurements were made on water extracts of the soil. These were made by thoroughly mixing 1 part of moist soil with 2 parts of distilled water and filtering through filter paper. This

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[3] MCCOOL, M. M., and MILLAR, C. E. OP. CIT.

[4] STEWART, Guy R. OP. CIT.

method gives results of the same relative values as those obtained by determining the total solids in water extracts or by estimates based on depressions of the freezing point in the soil itself. It is justifiable to assume that the conductivity measurements give at least a rough idea of the changes taking place in the soil solution under the various conditions.

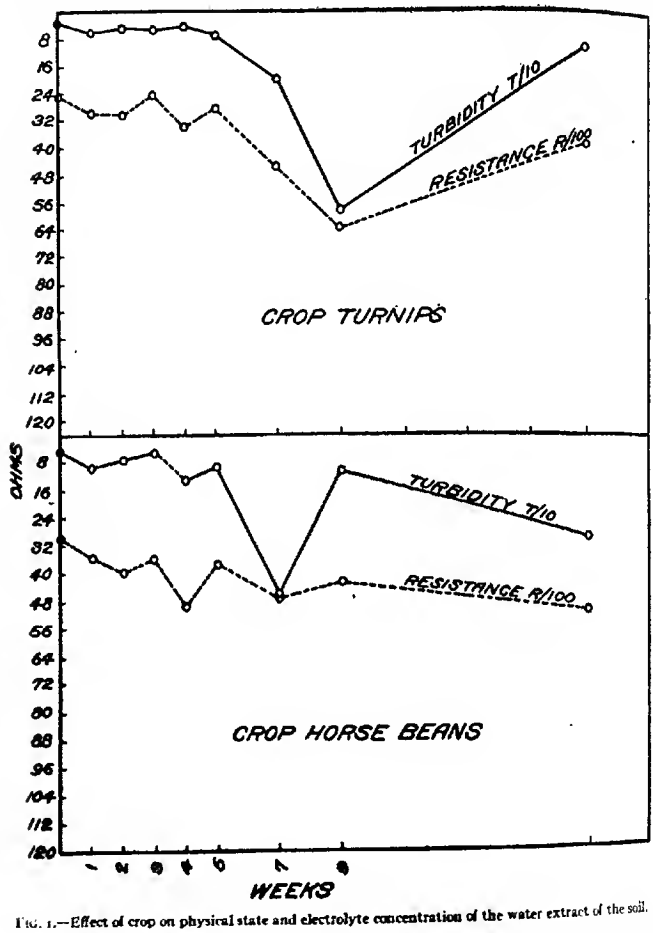

FIG. 1.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

Unfortunately no methods exist which permit the determination of the exact kinds or quantities of colloidal matter in the soil. We can measure only approximately certain resultant effects by the use of empirical procedures. Of these, turbidity observations are doubtless as valuable as any others. In the present experiments the samples of soil were mixed with water, in the proportion of 1 part of soil to 2 parts of water, and the

soil suspensions were poured into burettes. After 24 hours the upper 10 cc. were carefully pipetted off into weighed dishes, and the total residue was estimated after evaporation and drying at 100° C. While such a method unquestionably leaves much to be desired, it is nevertheless apparent that considerable changes in the colloidal state of the finer

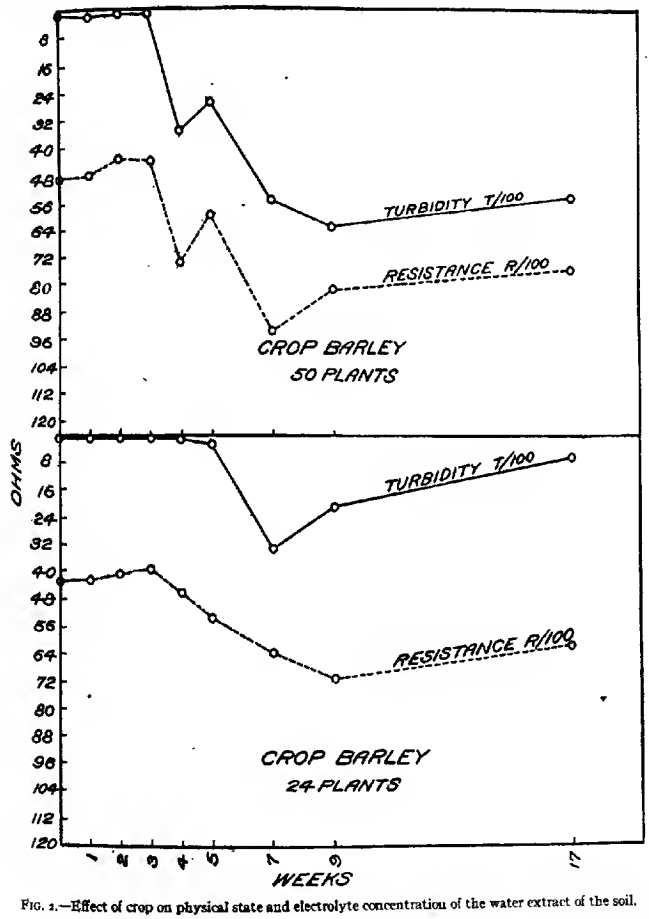

FIG. 2.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

soil particles are reflected in the quantities of suspended material obtained in this manner.

The data have been expressed in the form of graphs with the time (in weeks) plotted against values expressing the magnitudes of the suspended material and also against the resistances of the extracts in ohms. Since the concentration of the solution varies inversely as the resistance, the scale has been inverted to bring out the relations more clearly. (Fig. 1–4.)

It is evident that there exists a very good general correlation between the quantity of soluble constituents in the soil and the quantity of suspended material and that in both cases the magnitudes undergo very marked variations coincidentally with seasonal changes and crop growth. These fluctuations are far more pronounced, however, in the cropped

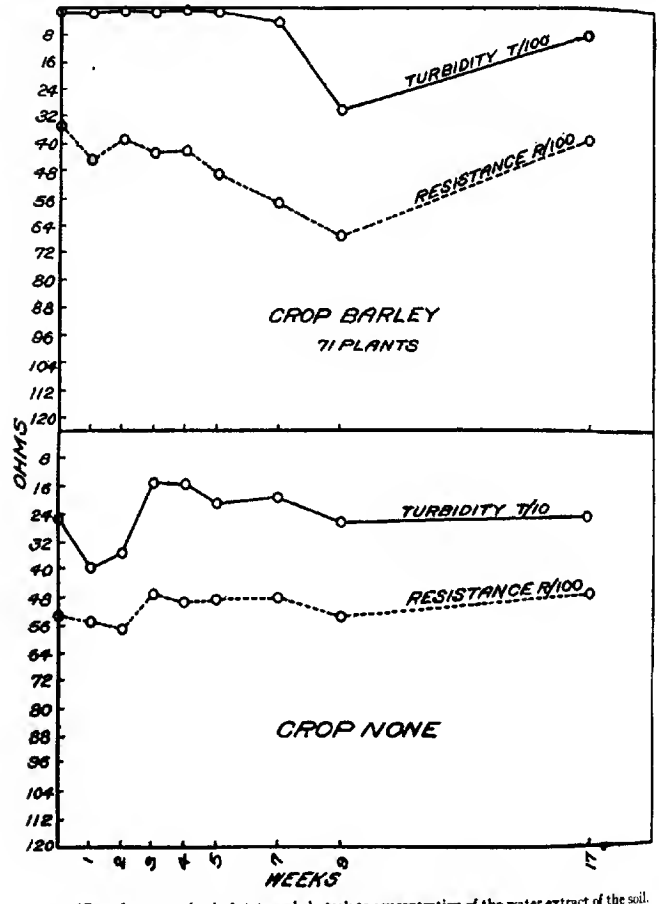

FIG. 3.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

soils than in the uncropped soils. In other words, it is a fair conclusion that the absorption of solutes by the plant has lowered the concentration of the soil solution at a period of 8 or 10 weeks after planting and that the physical state of the soil has undergone an equally definite change. It can scarcely be doubted that there is some definite relation between the concentration and composition of the soil solution and the

physical state of the soil. That this correlation is only approximate is not difficult to explain, even if we assume that the factors mentioned above are the only ones to be considered. The quantity of suspended material obviously can not bear an exact relation to the concentration of the solution throughout all ranges. At a certain point the supernatant liquid will become almost clear, and while further increases in the con

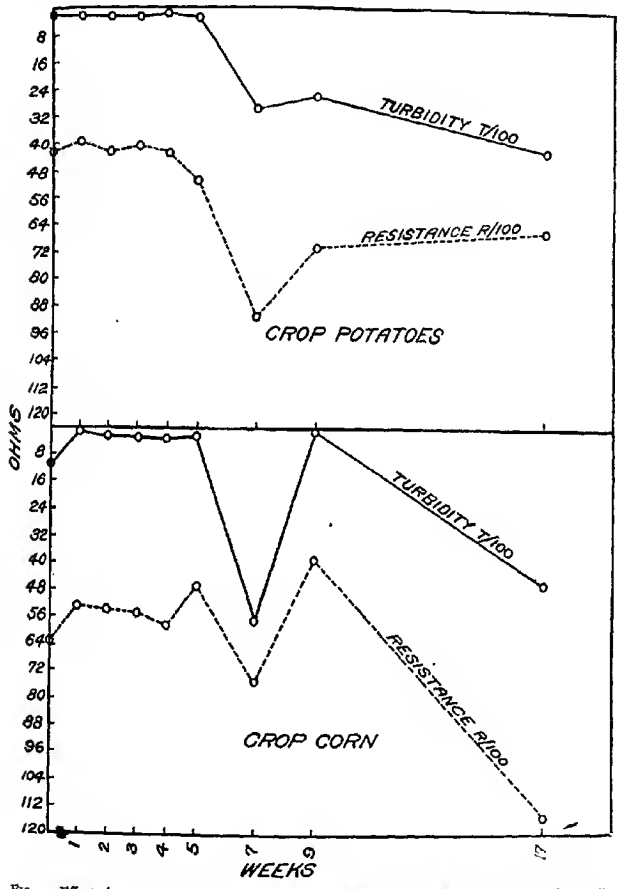

FIG. 4.—Effect of crop on physical state and electrolyte concentration of the water extract of the soil.

centration of electrolytes will diminish the resistance almost proportionally, no further change can be shown in the physical state of the soil as measured by the present method. Moreover, the conductivity is a resultant measurement expressive of the concentration or mobility of all the ions present. It is not true, however, that equivalent quantities of different ions have equal effects on the colloidal state of the soil.

The effect of various salts on the flocculation of soils has been studied in many investigations. Sharp [1] in an extensive series of observations has presented evidence to show that a remarkable change is produced in the physical state of the soil by the addition of various salts and subsequent washing of the soil with water. This change in the degree of dispersion of the colloids is attributed to the formation of new silicate compounds which give to the soil its new properties.

These investigations by Sharp have all dealt with rather extreme salt effects, such, for example, as might occur in "alkali" soils or heavily fertilized soils. So far as known, no study has been made of the changes which may take place in soils because of the normal fluctuations in the soil solution under varying conditions of cropping and season. In such cases the total quantity of salts dissolved in the soil solution is extremely small, and it might be questioned whether these could have any appreciable effect on the physical state of the soil. However, an analysis of the data presented by Stewart [2] and Hoagland [3] brings out the fact that relatively enormous fluctuations may take place in the soil solution. The growth of a crop, for example, in certain instances may reduce the concentration of the soil solution to an extremely low point. Recently McCool and Millar [4] have presented extensive data to show that in the field very profound changes may occur in the soil solution as a result of cropping, moisture variations, biological activities, rainfall, etc. Apparently all of these fluctuations in the soil solution may be reflected in the physical state of some at least of the soil constituents.

Since the effect of cropping is to reduce the water-soluble constituents of the soil and the concentration of the soil solution, it might be predicted on the basis of the foregoing discussion that soils which had been cropped would show a physical condition distinctly different from the same soils kept uncropped. In order to decide this point more definite turbidity determinations were made on a number of different soils. Except that one tank of each soil had been cropped for four years and one tank had been kept without crop for three years, the soils were maintained under identical conditions. Originally both portions of the soil were from one sifted, homogeneous mass. The details of treatment have already been described in an article by Stewart.[5] Chemical analyses and conductivity measurements on water extracts, as well as freezing-point depressions on the moist soil, all pointed to the fact that the uncropped soil yielded a soil solution of higher concentration than did the cropped soil. The data contained in Table I give evidence that these differences were reflected in the physical state of the soils. It is particularly easy to demonstrate this relation for the silty soils, but even the sandy soils display the same tendency.

[1] SHARP, L. T. OP. CIT.
[2] STEWART, Guy R. OP. CIT.
[3] HOAGLAND, D. R. OP. CIT.
[4] MCCOOL, M. M., and MILLAR, C. E. OP. CIT.
[5] STEWART, Guy R. OP. CIT.

TABLE I.—*Relation of physical state to the electrolyte concentration of the soil extract*

Soil No.	Condition of soil.	June 3.		July 26.	
		Turbidity.[a]	Specific resistance.	Turbidity.[a]	Specific resistance.
			Ohms.		*Ohms.*
1	Cropped	170	4,500	.....	.....
	Uncropped	80	2,900	.....	.....
2	Cropped	330	5,000	1,400	6,800
	Uncropped	190	4,200	640	5,100
3	Cropped	230	4,300	6,620	6,900
	Uncropped	160	3,700	220	4,100
4	Cropped	170	4,300	100	3,900
	Uncropped	90	2,900	60	3,000
5	Cropped	290	4,800	410	5,800
	Uncropped	100	2,700	160	4,100
6	Cropped	270	4,500	.....	.....
	Uncropped	180	3,400	.....	.....
7	Cropped	1,120	6,500	.....	.....
	Uncropped	950	5,900	.....	.....
8	Cropped	340	5,800	.....	.....
	Uncropped	300	3,700	.....	.....
9	Cropped	1,090	8,000	.....	.....
	Uncropped	1,070	7,500	.....	.....
10	Cropped	970	7,400	1,060	10,400
	Uncropped	1,330	7,000	610	4,400
11	Cropped	1,730	5,800	2,100	5,900
	Uncropped	130	3,300	160	3,400
12	Cropped	1,220	12,400	1,320	16,900
	Uncropped	1,050	6,500	1,160	8,100
14	Cropped	1,320	8,700	1,260	9,200
	Uncropped	1,140	6,300	1,420	6,500
16	Cropped	.....	.....	2,010	9,000
	Bin	.....	.....	150	4,300

[a] Expressed in milligrams per 100 cc.

It has already been pointed out that under certain conditions of storage a soil may accumulate a large amount of soluble constituents. It was thought to be of interest to compare a sample of soil which had been kept in a bin for several years in a slightly moist condition with a sample of the same soil cropped for several years. The two samples displayed widely different concentrations of electrolytes, and the turbidity measurements indicate that 20 times as much material was kept in suspension in the cropped soil. These samples demonstrate the extreme effects which may occur, even without fertilization or leaching.

Sharp[1] has shown that salt-treated soils washed with water are made far more impervious than soils washed with water without previous treatment. If, however, a soil is very completely leached with distilled water after stirring, an extremely impervious condition of the soil results. At the same time the final leachings are exceedingly dilute, and the concentration of solution in the leached soil is so small as to be scarcely determinable. In Sharp's experiments the impervious condition of the

[1] SHARP, L. T. *op. cit.*

soil is considered to be the result of the formation of certain new silicates. Possibly in soils leached with water and not containing an excess of salts the dispersed condition may be the result of the almost complete removal of electrolytes from the films of solution surrounding the soil particles. To a lesser extent the same thing occurs when the soil solution is depleted through absorption of solutes by the plant. None of the data presented in this paper, however, are of such a nature as to permit of any conclusions with regard to these very difficult questions concerning the colloid chemistry of the soil.

Neither is it possible to state definitely the effects of the fluctuating soil solution on the physical state of the soil under field conditions. A sample of soil may be maintained in a relatively pervious state even after long washing, provided the compound particles of soil are not disturbed by stirring or mixing while the soil is saturated with moisture. Nevertheless, it is probable that the soil in the field is subject to certain modifications in its physical state which are merely accentuated when the laboratory tests are carried out.

It is interesting to speculate on the indirect effects of the changes in the physical condition of the soil noted in these experiments. It is entirely possible that such changes may be of considerable importance. The aeration, resistance to root penetration, ease of cultivation, percentage of unfree water, etc., are very probably affected to a greater or less degree, and these alterations in the soil conceivably may have an important influence on the growth of microorganisms or plants. In any case, it is highly desirable to make observations on all the effects, direct and indirect, which may be correlated with the changing concentration or composition of the soil solution. It should be strongly emphasized that in studies of soil fertility the whole system of soil, soil solution, and plant is so constituted that all the components must be considered as interrelated. Thus, the plant may exhaust the soil solution with a resultant change in physical condition of the soil which may be unfavorable to the growth of microorganisms, and this inhibition in time may influence the concentration of certain solutes in the soil solution. It is believed that the greatest advances in theories of soil fertility will come with an extension of our knowledge of the soil solution in its dynamic aspects.

CONCLUSIONS

The physical state of certain soil constituents is influenced to a marked degree by the concentration of the soil solution. The colloidal state of the soil suspension undergoes significant alterations during the season. A large increase in colloidal matter is noted when the soil solution is depleted as a result of absorption of solutes by the plant.

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